Title. Complete characterization of the seventeen step moenomycin biosynthetic pathway

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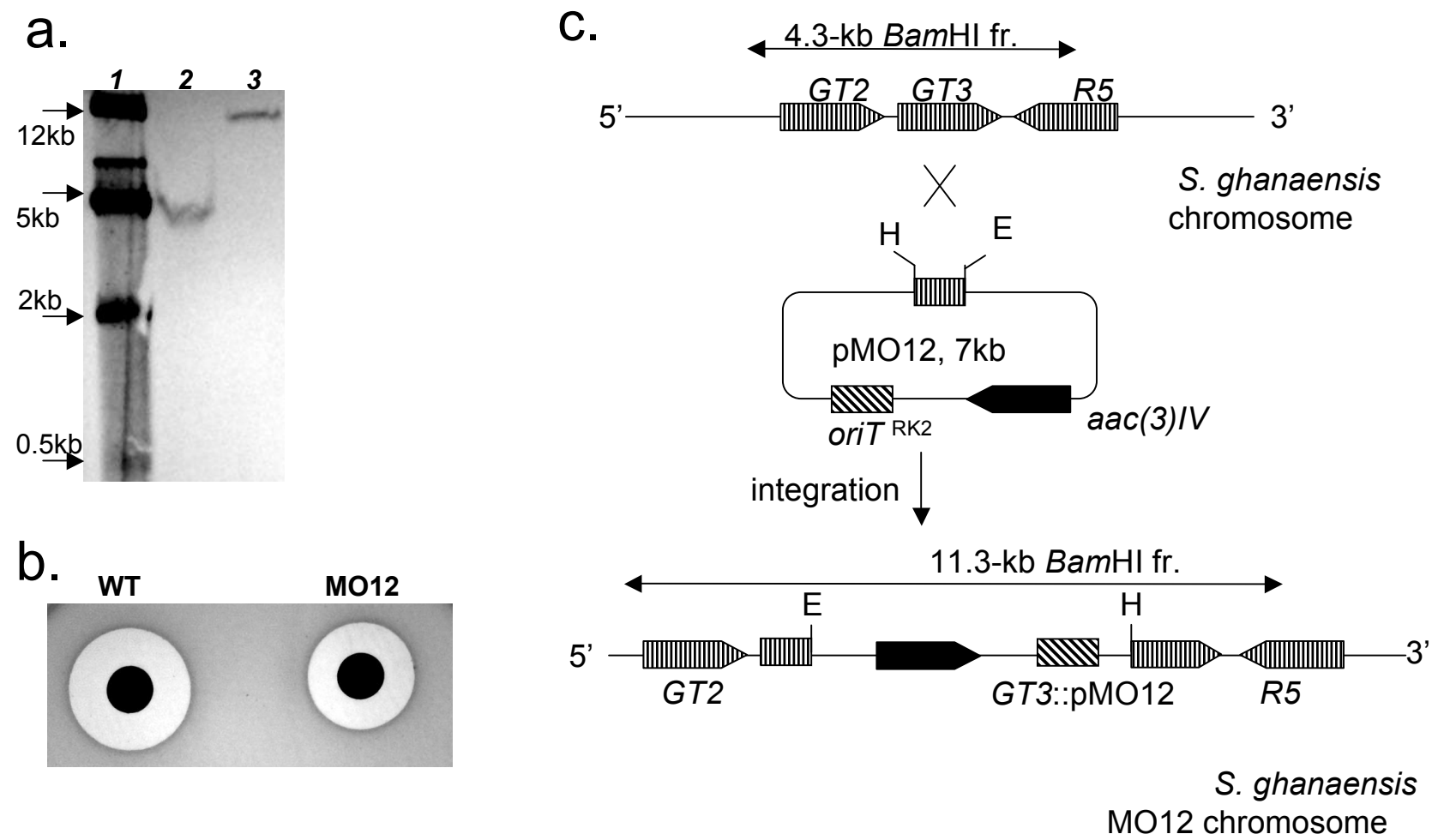


Figure S1. Generation of the *S. ghanaensis* MO12 strain **a.** Southern analysis of *Bam*HI digests of total DNA of wild type *S. ghanaensis* (lane 2) and MO12 strain with disrupted *moeGT3* (lane 3). Lane 1 – mixture of plasmids pMO12, pMO14 and pOOB58 underdigested with *Pst*I. **b**. Bioassay of semipurified extracts from 1g (wet weight) of mycelia of wild type strain (WT) and MO12. **c.** Scheme of *moeGT3* disruption in the *S. ghanaensis* genome. H and E mark *Hin*dIII and *Eco*RI sites, respectively.

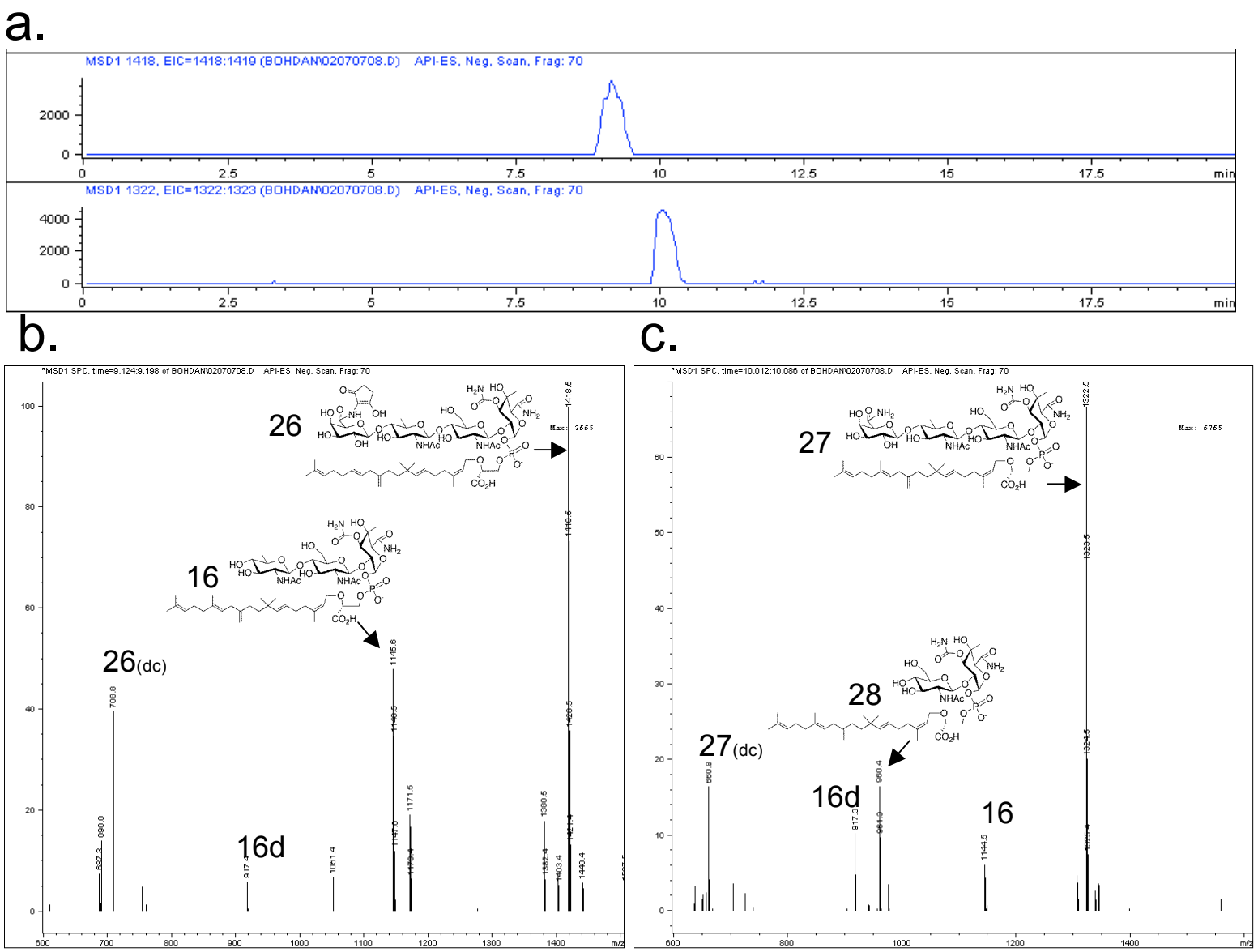
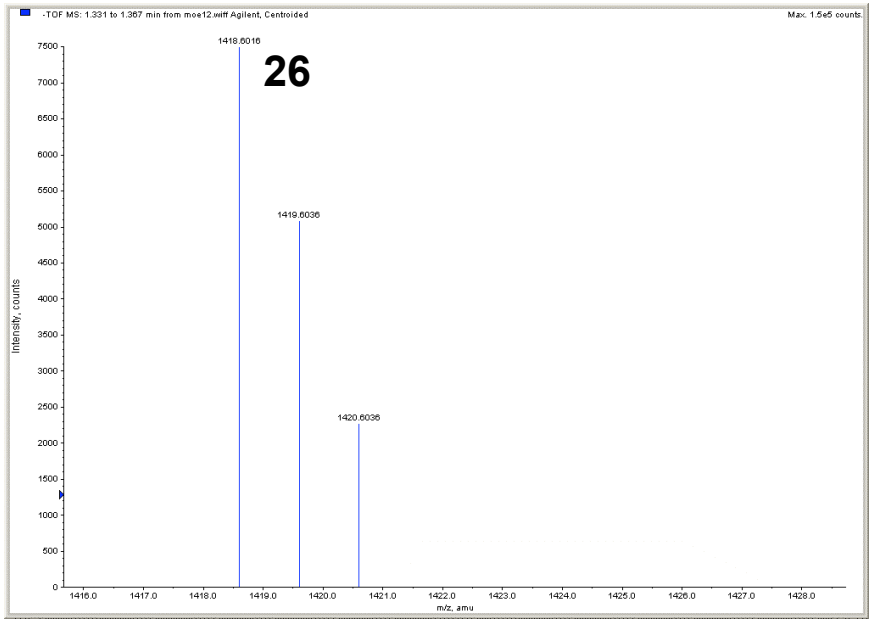


Figure S2. LC-MS analysis of moenomycin metabolites accumulated by the *S. ghanaensis* MO12 strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is moenomycin C_4 (**26**) having Rt 9.2 min. **c.** The strain also accumulates its precursor lacking the A ring (**27;** Rt 10.0 min). Peaks corresponding to trisaccharide and disaccharide fragments of moenomycin C_4 (**16** and **28**, respectively) are observed. **16d** is the decarbamoylated derivative of **16**. **26(dc)** and **27(dc)** are doubly charged ions of **26** and **27**, respectively.

a.



b.

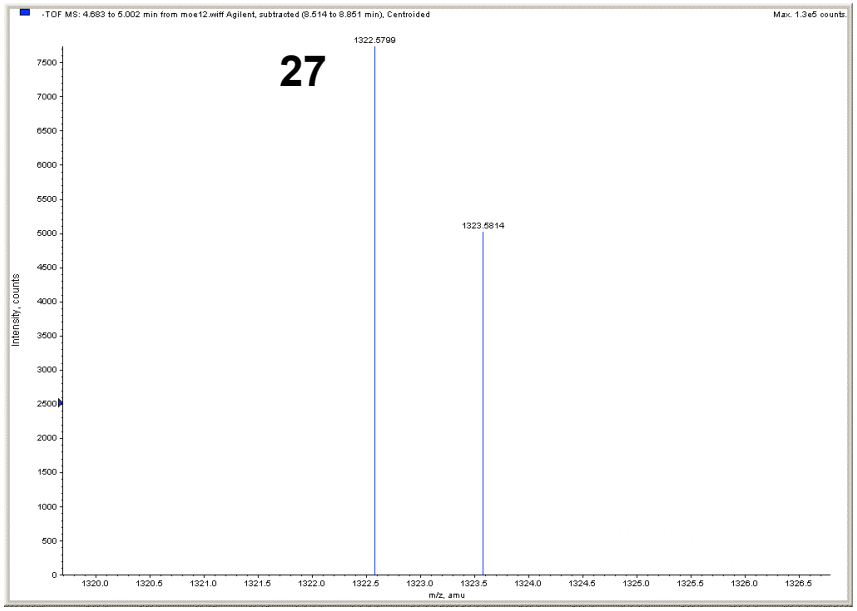


Figure S3. LC-MS exact mass analysis of moenomycin metabolites accumulated by the *S. ghanaensis* MO12 strain. Instrumentation and conditions are described previously. **a.** The final product is moenomycin C_4 (**26**) **b.** The strain also accumulates its precursor lacking the A ring (**27**).

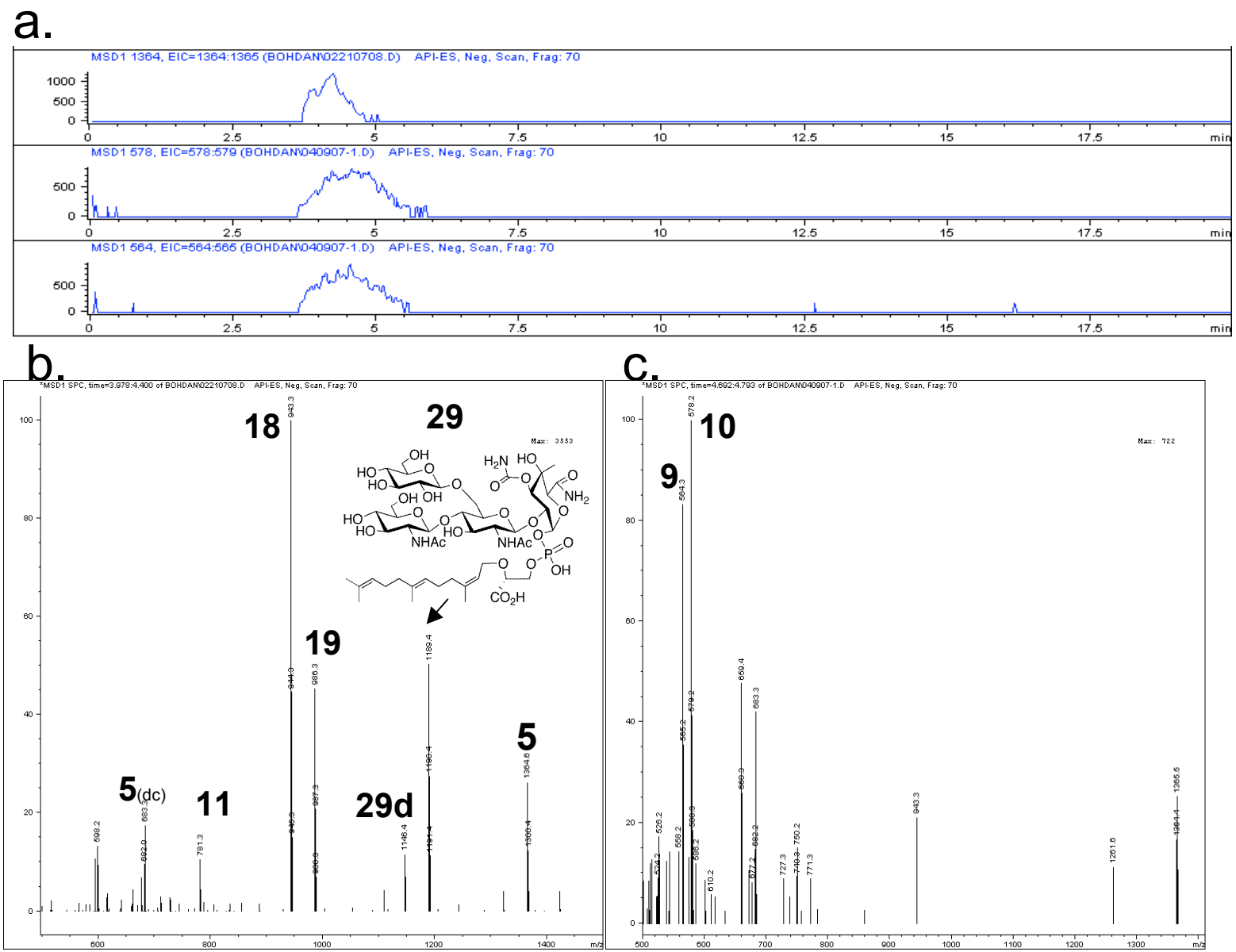


Figure S4. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ*moeN5* strain. **a.** Selected ion chromatograms of the purified extracts. . **b.** The final product is compound **5** having Rt of 4.2 min. **c.** The strain also accumulates monosaccharide precursors **9** and **10** (Rt 4.7-4.8 min). Structure of **5** is shown in **Fig. 1b** and structures of **9, 10, 11, 18,** and **19** are shown on **Fig. 2**. **29d** is the descarbamoylated derivative of **29**. **5(dc)** is doubly charged ion of **5.**

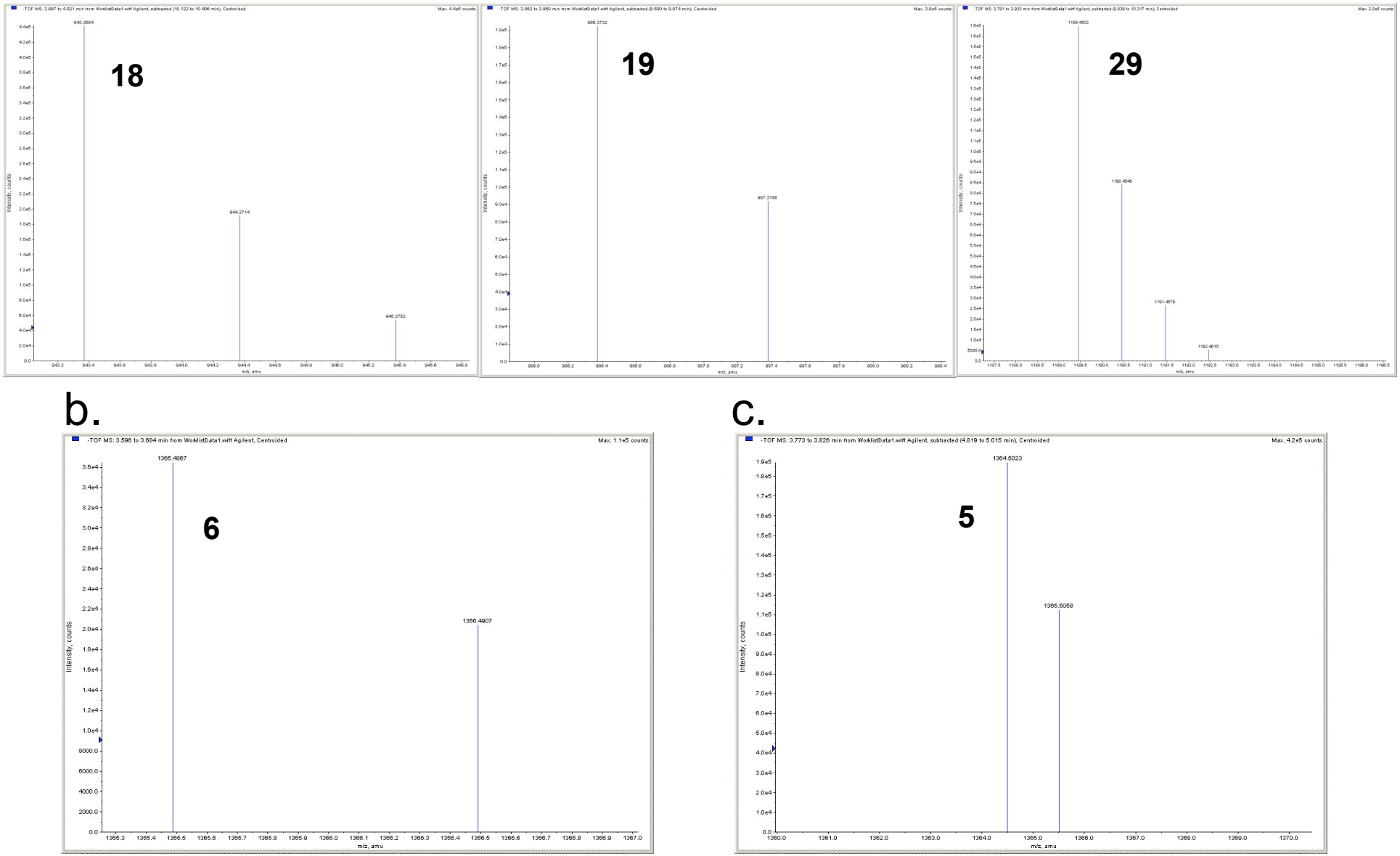
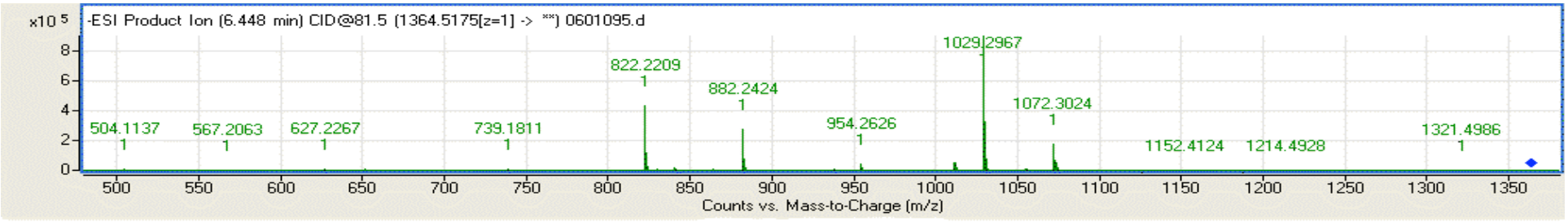
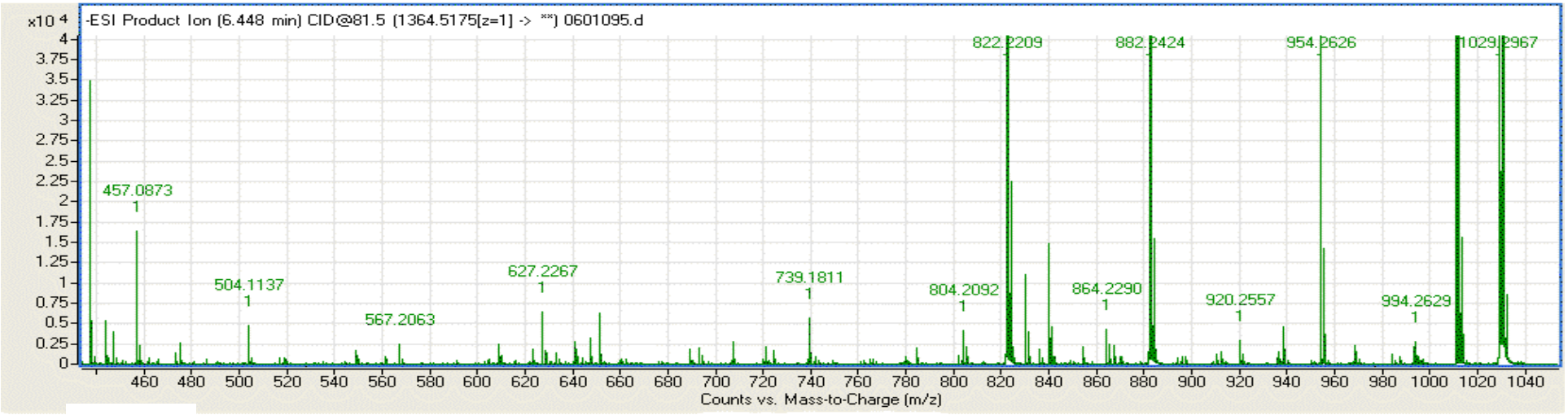


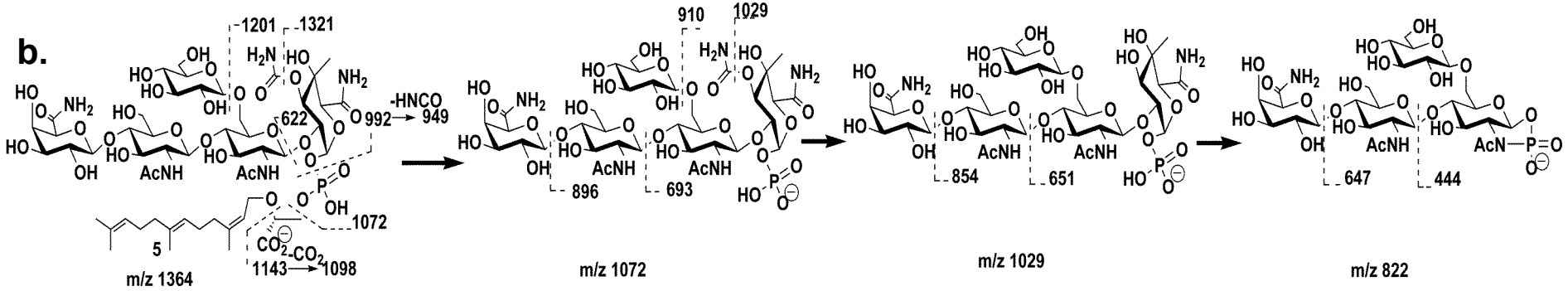
Figure S5. LC-MS exact mass analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ *moeN5* strain. Instrumentation and conditions are described previously. **a.** The exact mass of proposed intermediates **18, 19** and **29** were observed. The strain accumulates both the acid (**b. 6**) and the amide (**c., 5**) forms of the final C15 pentasaccharide.

a.



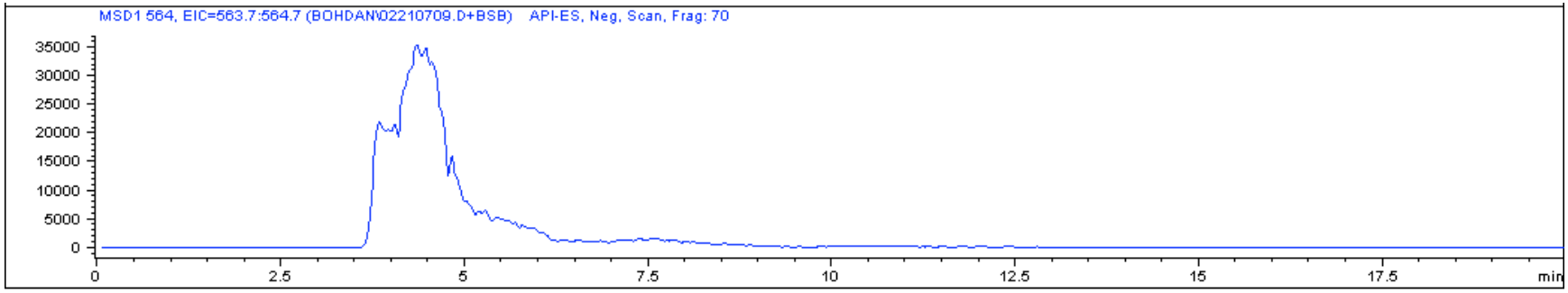


b.

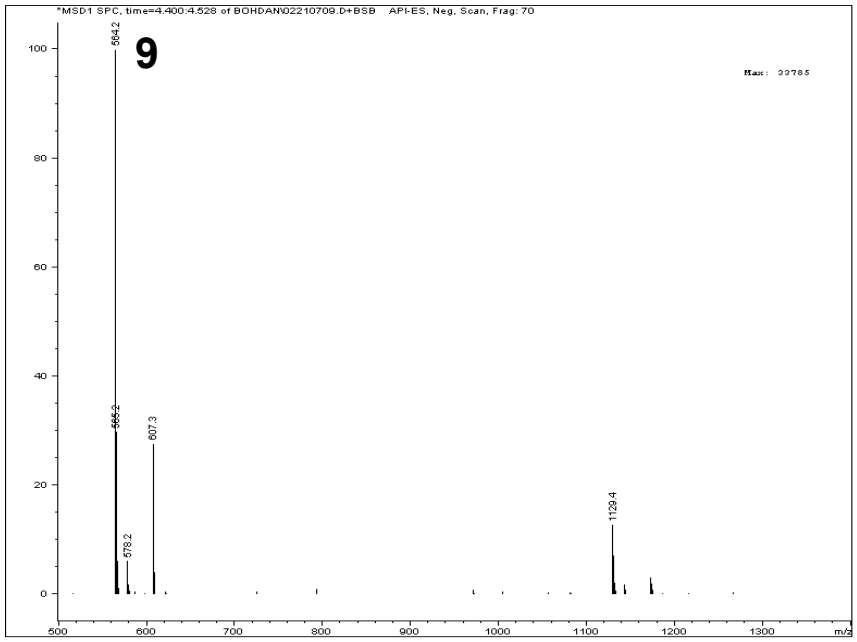


Supplementary Figure S6. a. ESI-MS2 spectrum of a mixture of compounds **5** and **6** produced by the *S. lividans* Δ*moeN5* strain. **b.** The observed fragmentation pathway of the compounds.

a.



b.



c.

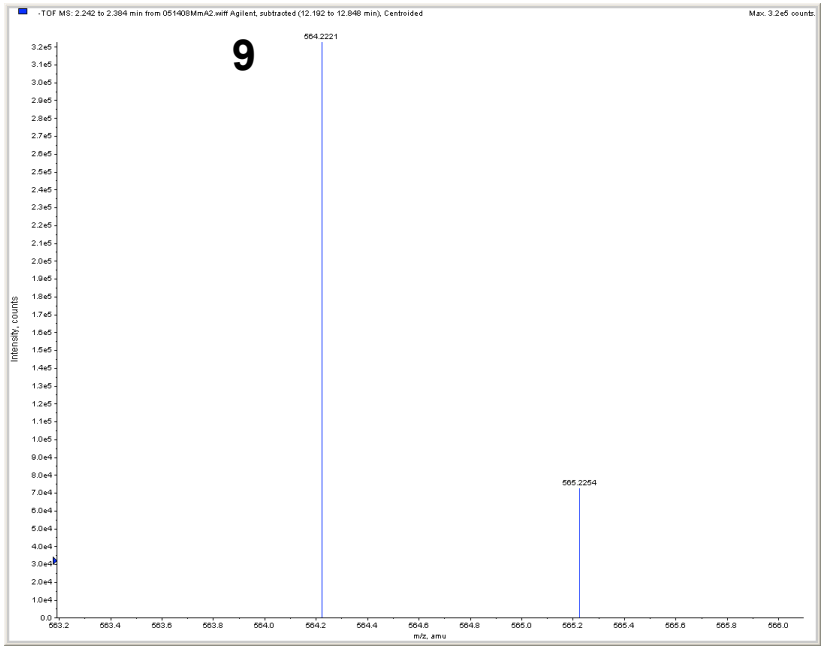


Figure S7. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ *moeGT4* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The strain accumulates compound **9** (Rt 4.7 min) or it's equatorial epimer. **c.** Exact mass analysis of compound **9.**

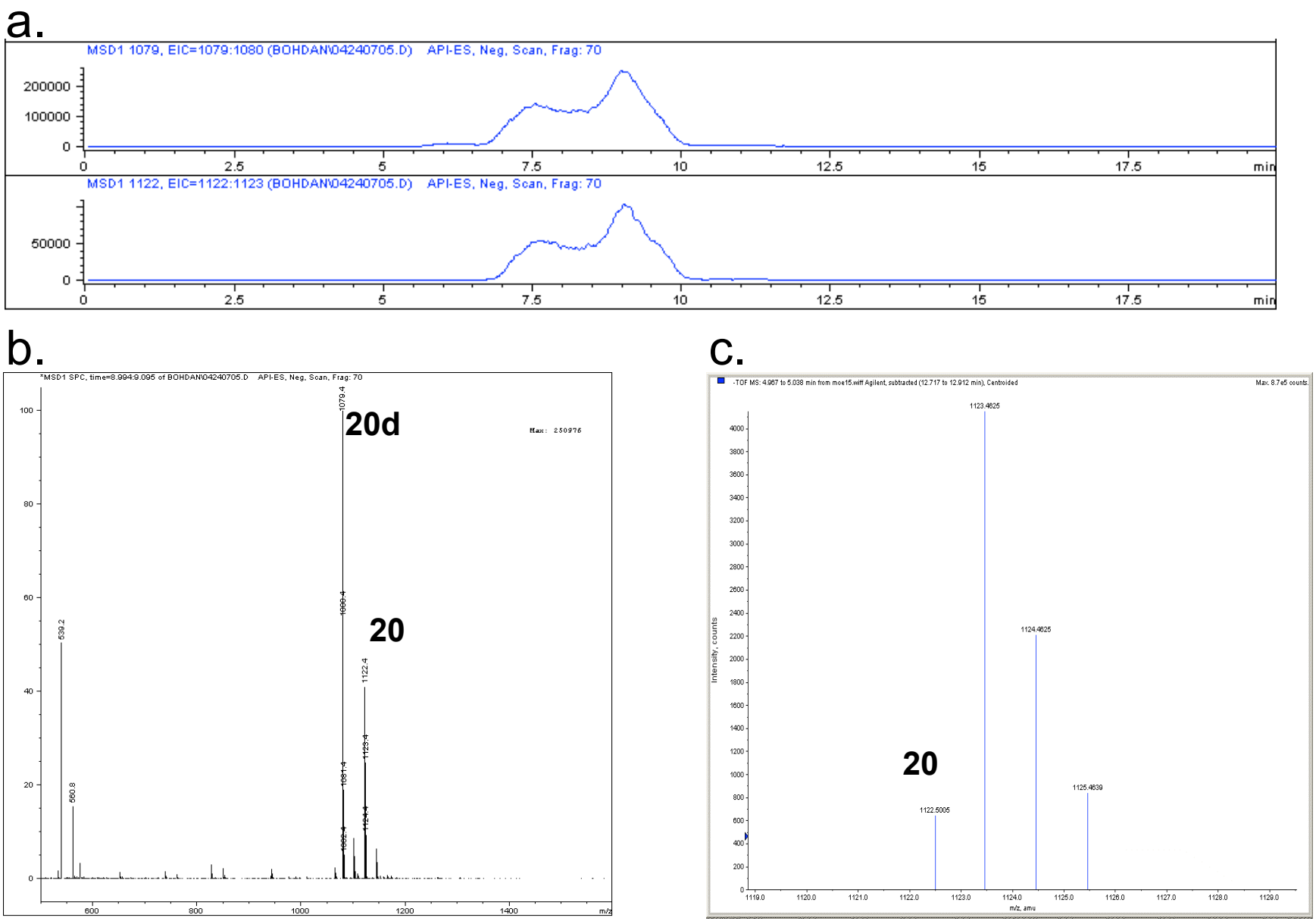
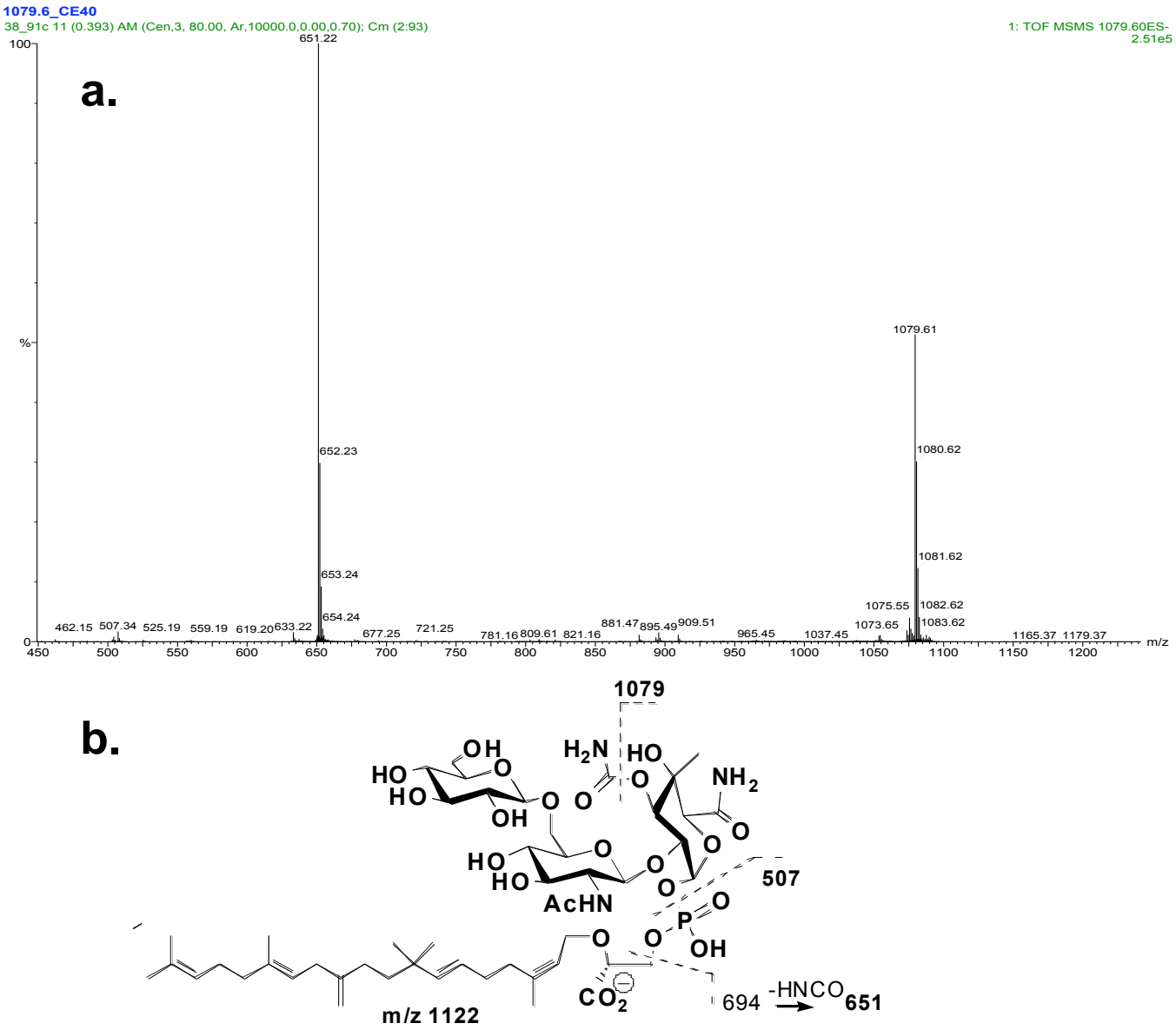
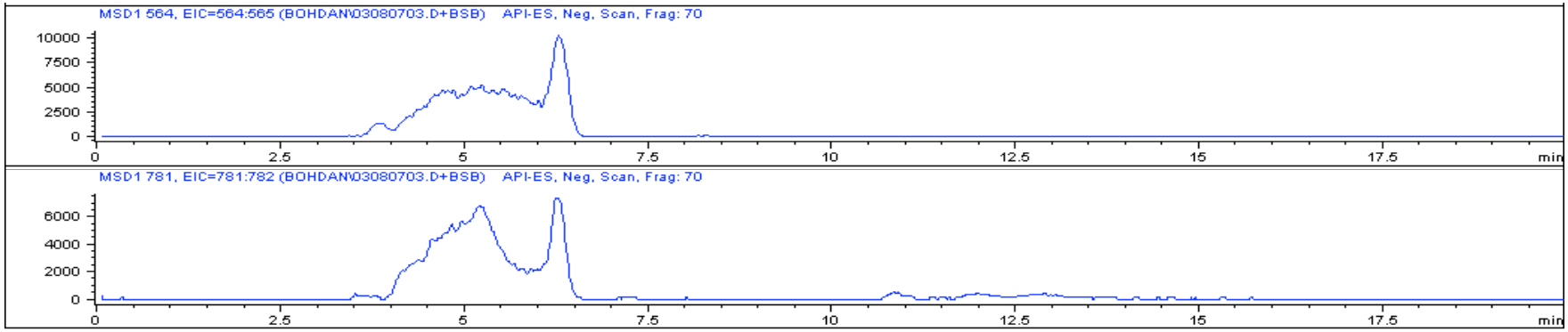


Figure S8. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ*moeGT5* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is compound **20** (Rt 10.4 min)**.** The strain also accumulates its descarbamoylated derivative **20d**. **c.** Exact mass analysis of compound **20**.

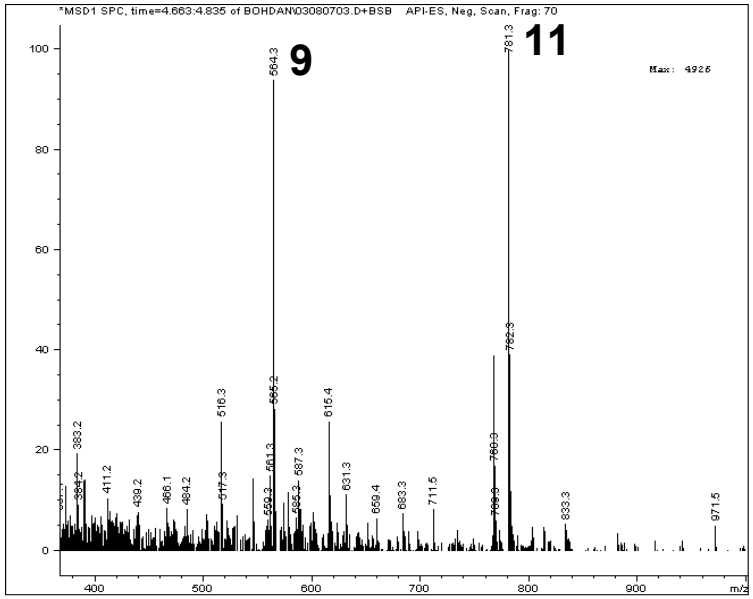


Supplementary Figure S9. a. ESI-MS2 spectrum of compound **20** (Rt 10.4 min) produced by the *S. lividans* Δ*moeGT5* strain. **b.** The observed fragmentation pathway of **20**.

a.



b.



c.

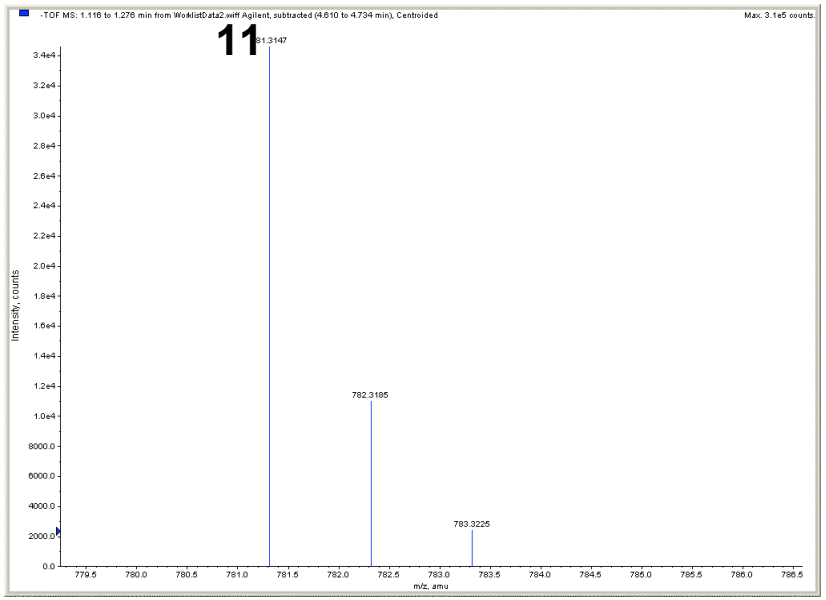


Figure S10. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ *moeGT5*Δ*moeGT3* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is compound **11** (Rt 4.8 min). The strain also accumulates its desmethylated precursor **9** (Rt 4.7 min) or equatorial epimer. **c.** Exact mass analysis of the final compound **11**.

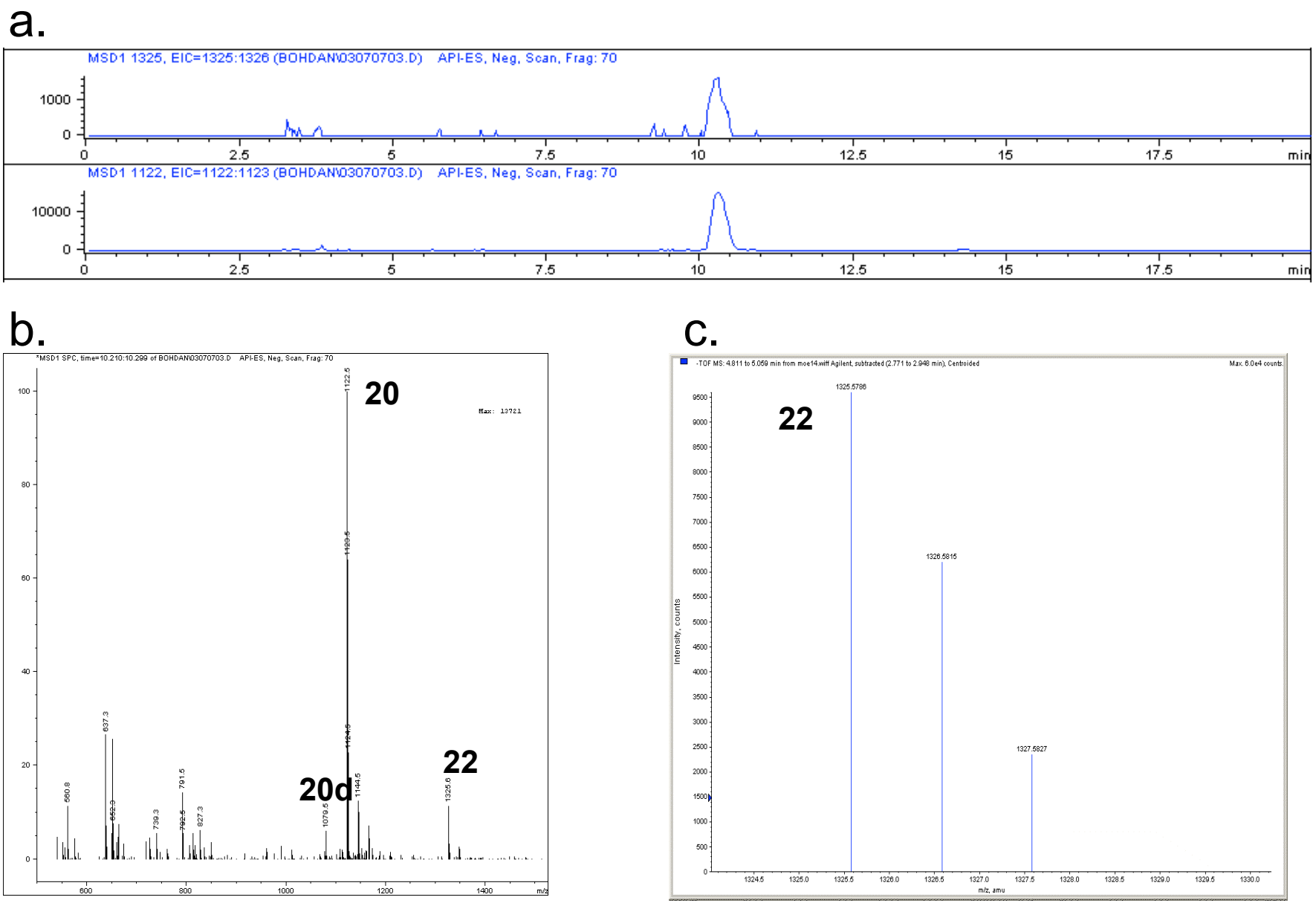
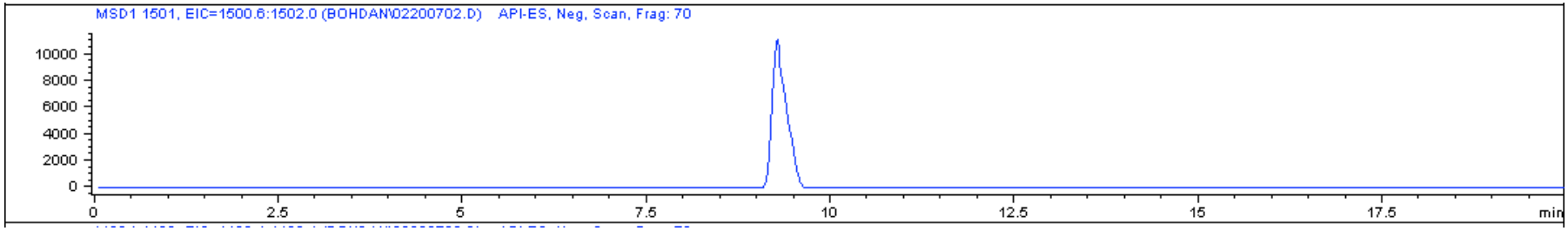
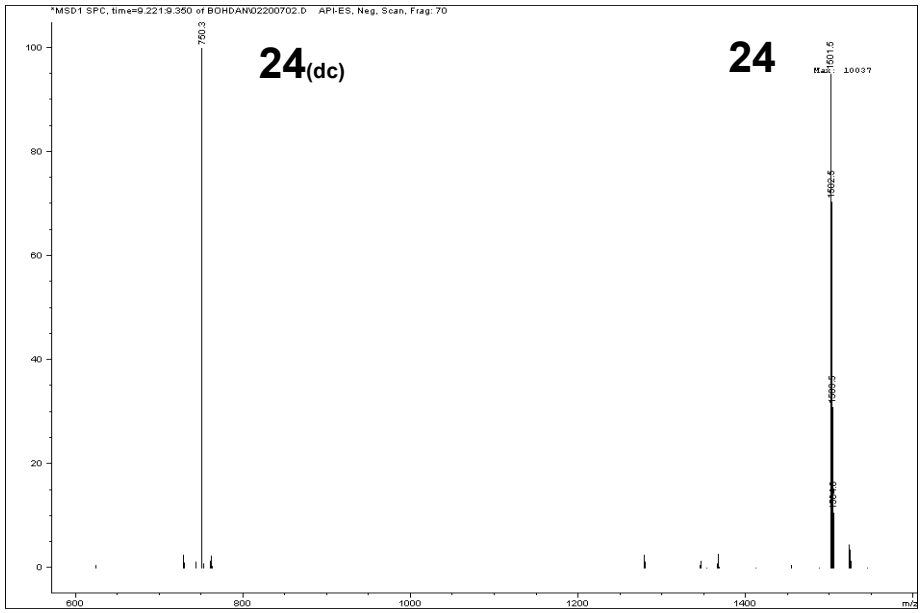


Figure S11. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ*moeGT2* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is compound **22** (Rt 10 min). The strain also accumulates the precursors to **22**: **20** and **20d**. **c.** Exact mass analysis of compound **22**.

a.



b.



c.

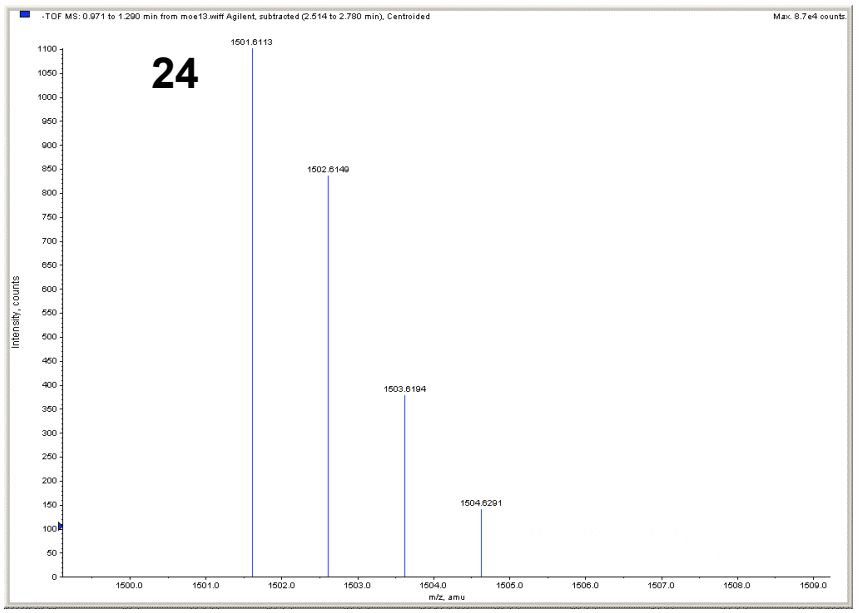
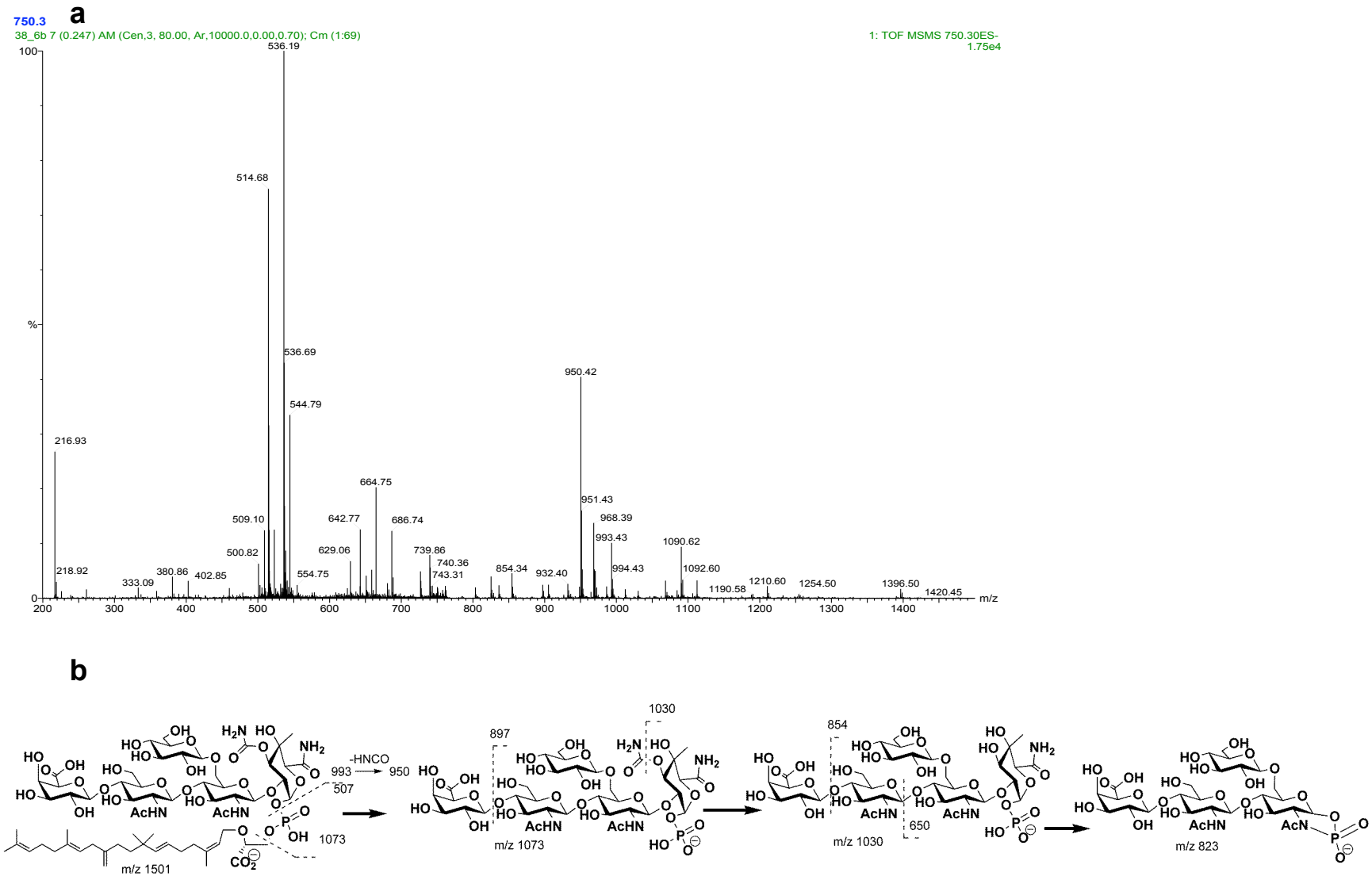


Figure S12. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ*moeH5* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is compound **24** (Rt 9.3 min). **24(dc)** is doubly charged ion of **24**. **c.** Exact mass analysis of compound **24**.



Supplementary Figure S13. a. ESI-MS2 spectrum of compound **24** produced by the *S. lividans* Δ*moeH5* strain. **b.** The observed fragmentation pathway of the compound **24**.

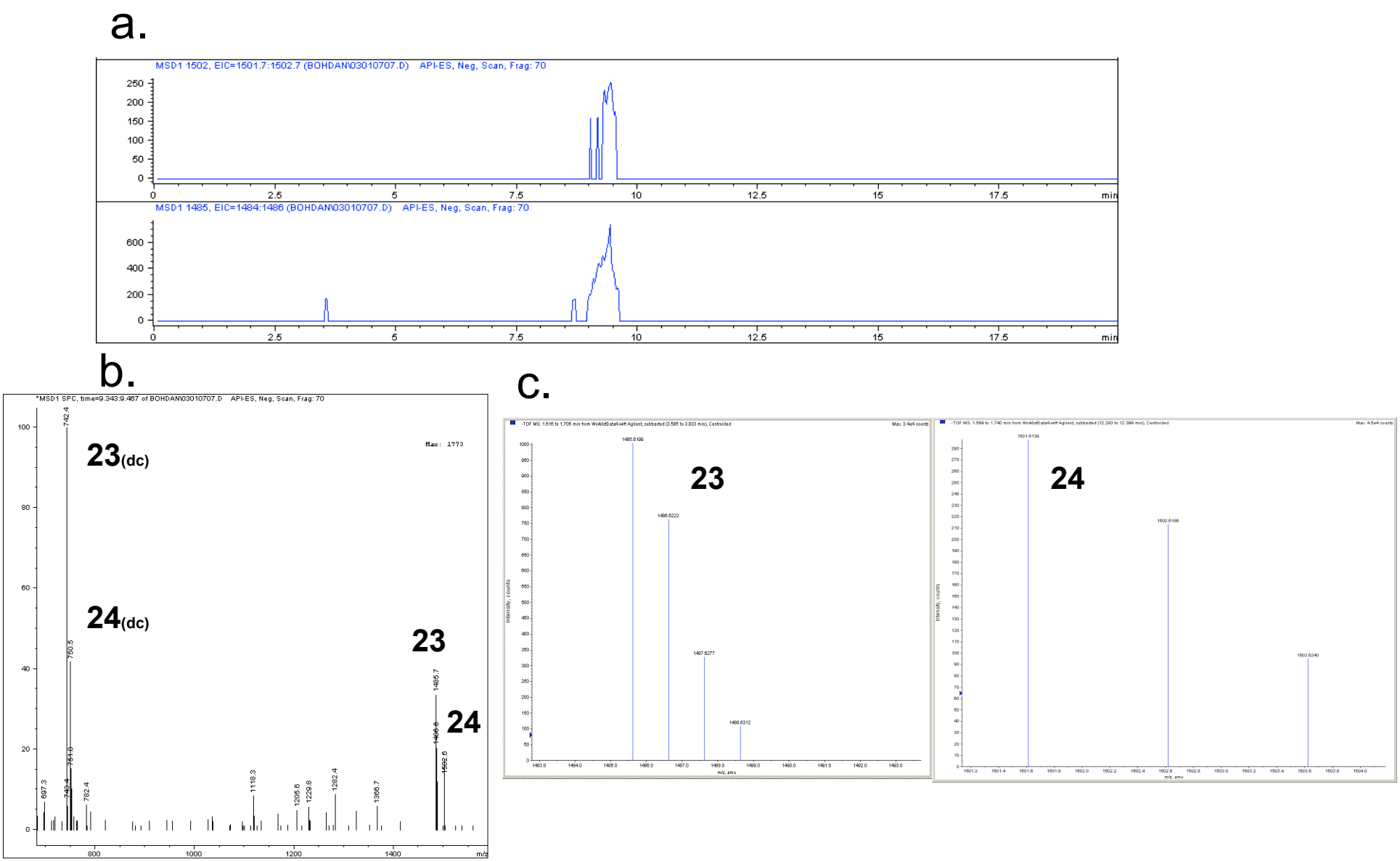
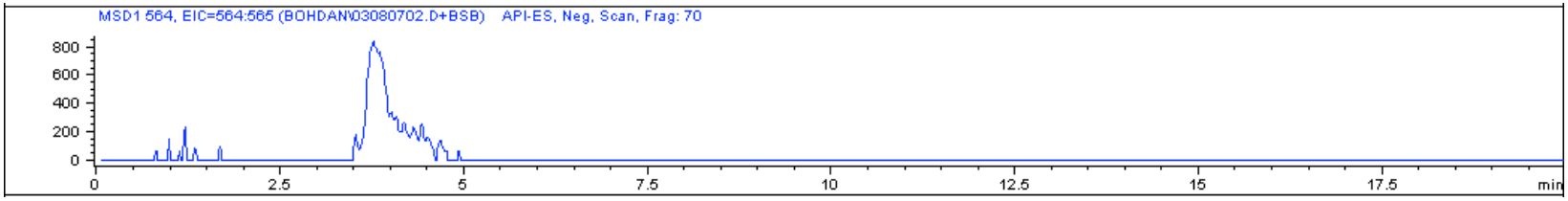
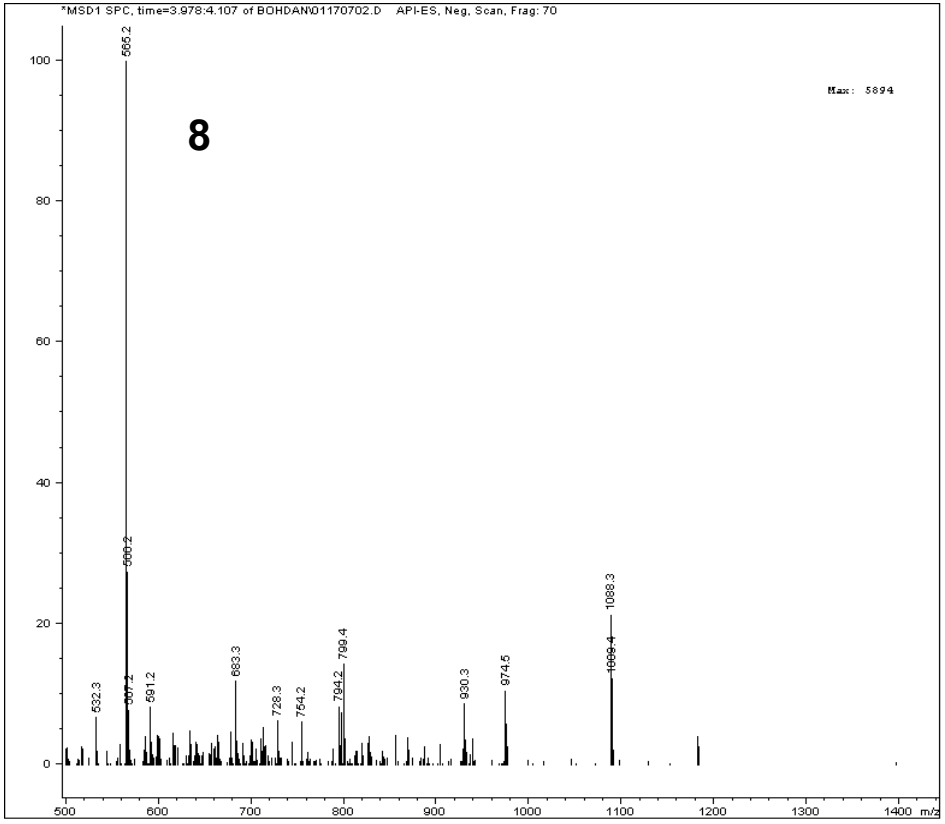


Figure S14. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ *moeH5* strain overexpressing the *moeR5* gene. **a.** Selected ion chromatograms of the purified extracts. **b.** The strain accumulates both **24** and **23**. **24(dc)** and **23(dc)** are doubly charged ions of **24** and **23**, respectively. **c.** Exact mass analysis of compounds **23** and **24.**

a.



b.



c.

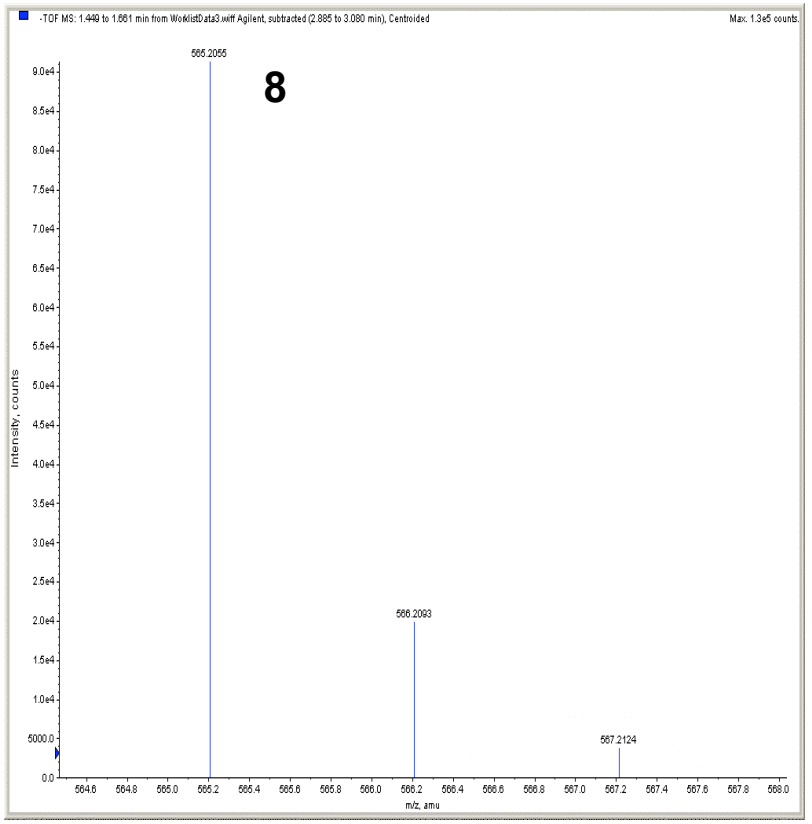
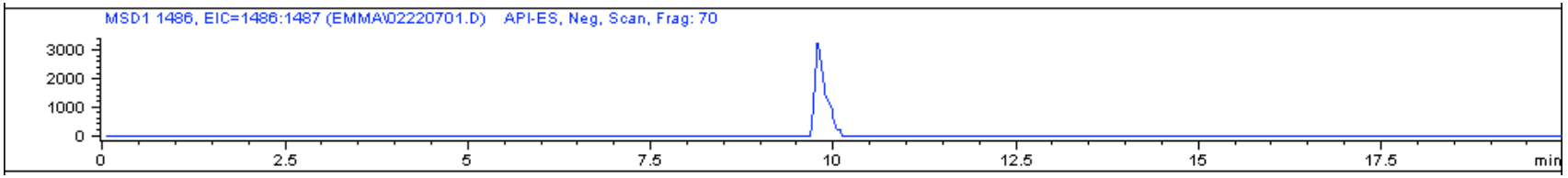
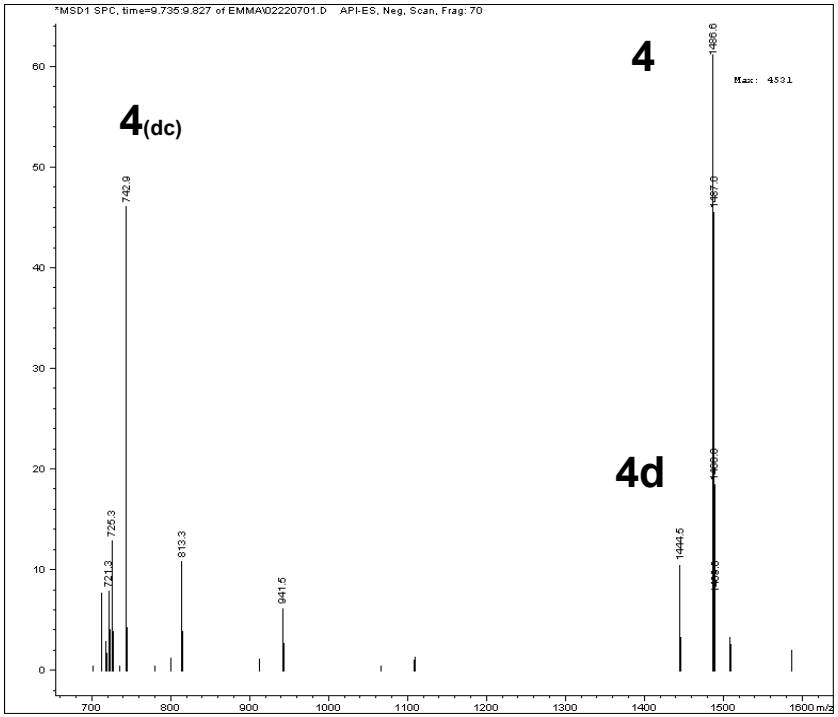


Figure S15. LC-MS analysis of moenomycin metabolites accumulated by *S. lividans* TK24 Δ*moeF5* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The strain accumulates compound **8** (Rt 3.9 min). **c.** Exact mass analysis of compound **8.**

a.



b.



c.

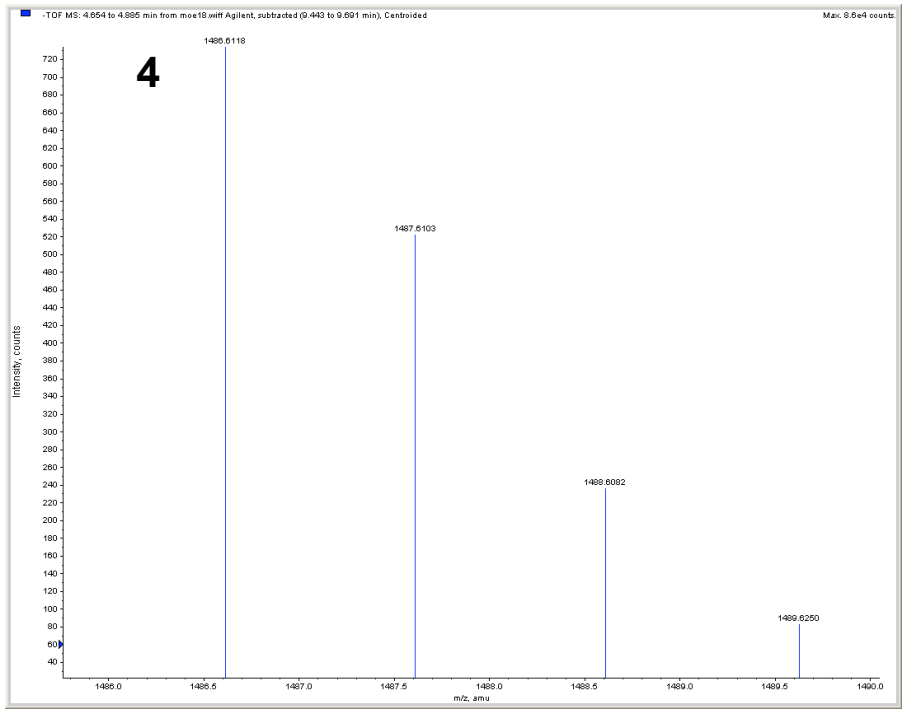
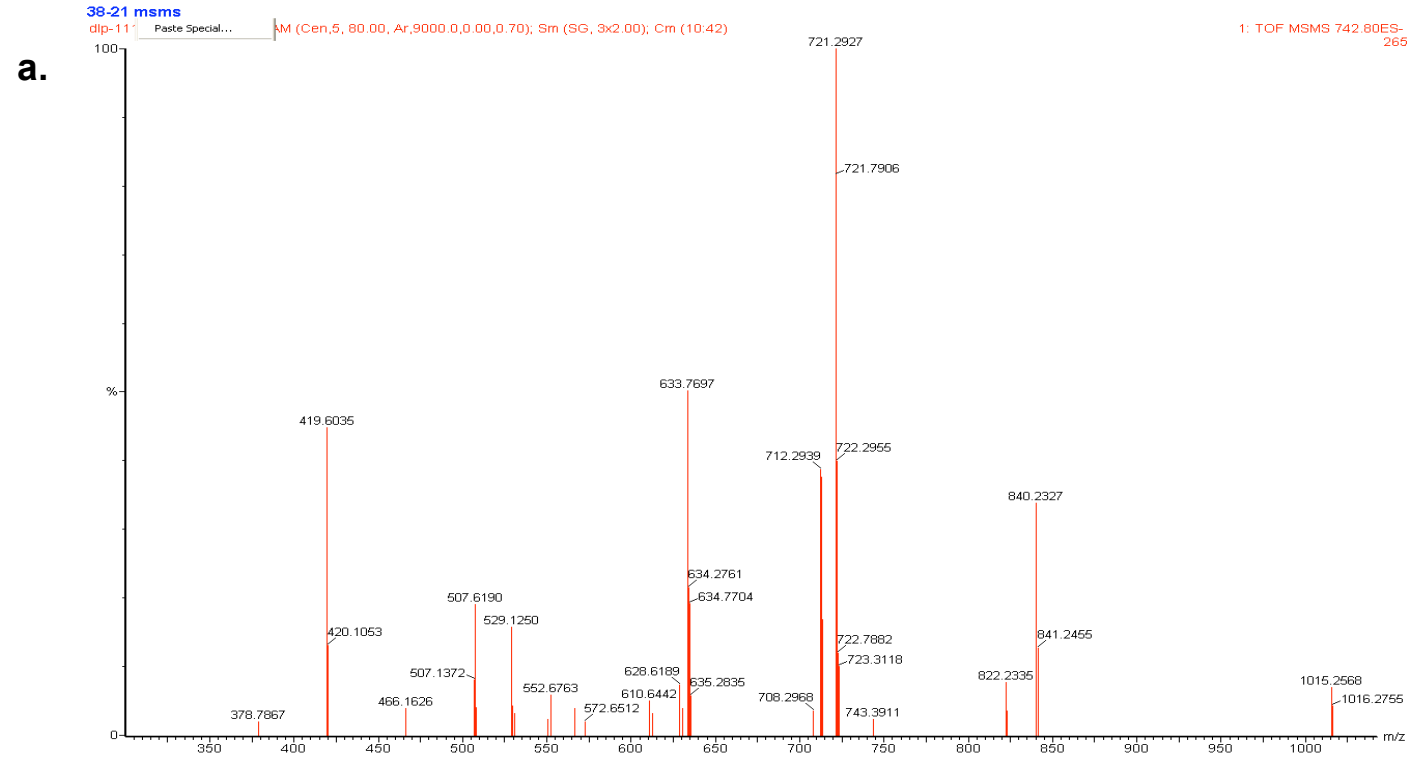
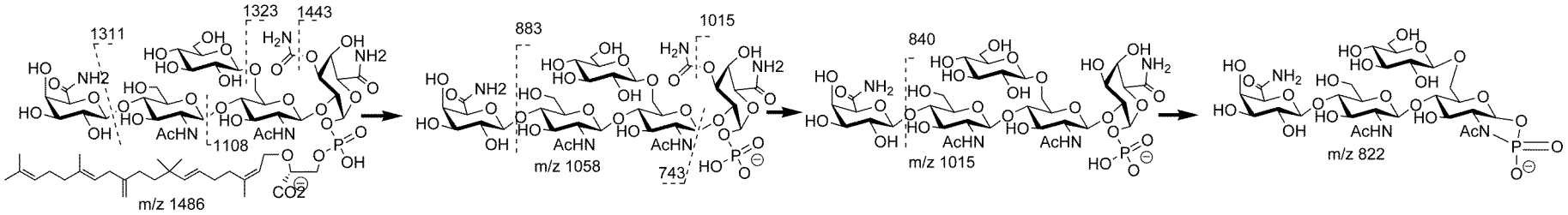


Figure S16. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ *moeK5* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The strain produces compound **4** (Rt 9.6 min), a demethylated analog of **3**. **6d** is the descarbamoylated analog of **4**; **4(dc)** is doubly charged ion of **4**. **c.** Exact mass LCMS analysis of compound **4**.

a.



b.



Supplementary Figure S17. a. ESI-MS^2 spectrum of compound **4** produced by the *S. lividans* Δ*moeK5* strain. (dc) following the ion label indicates a doubly charged species. **b.** The observed fragmentation pathway of the compound.

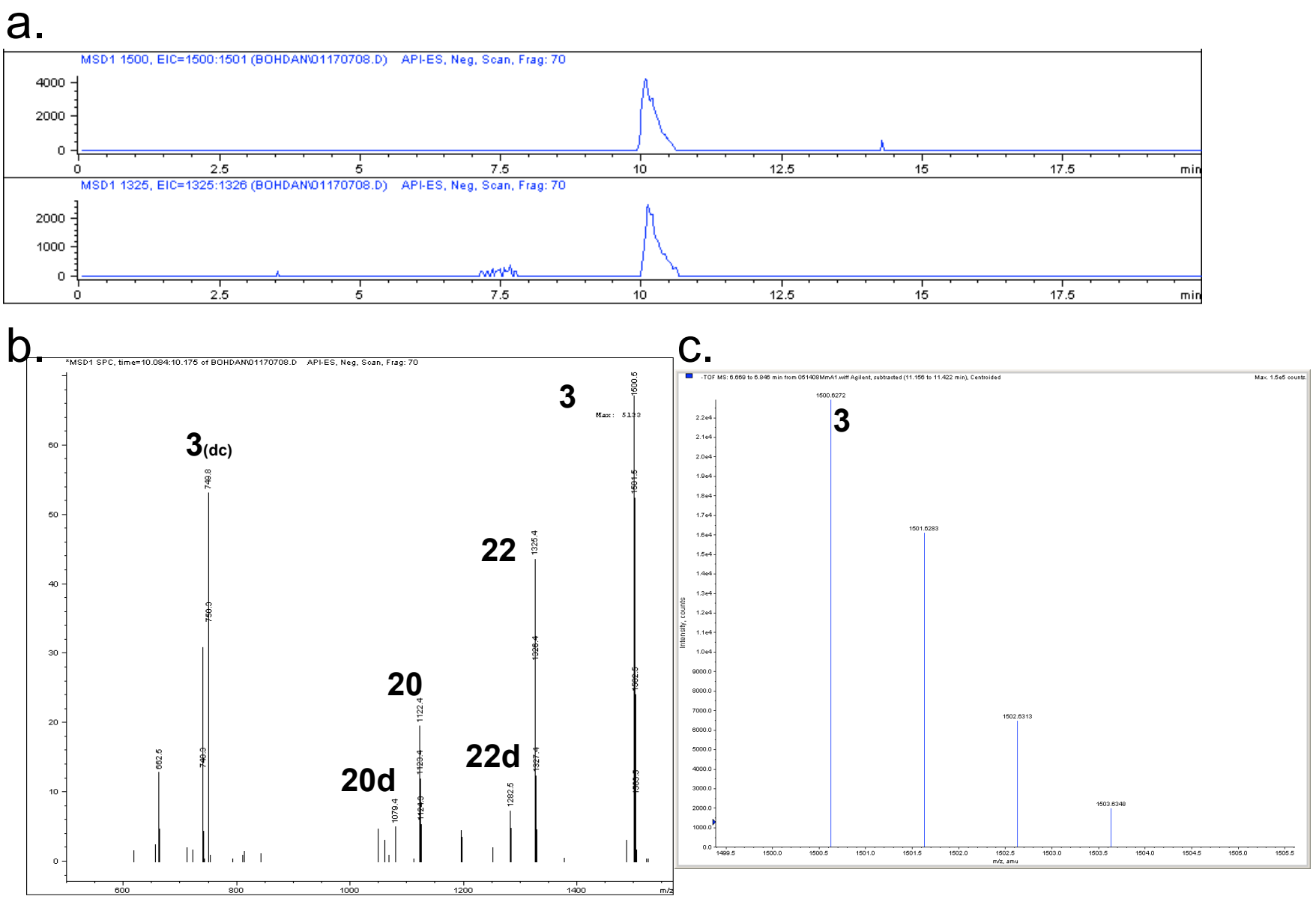
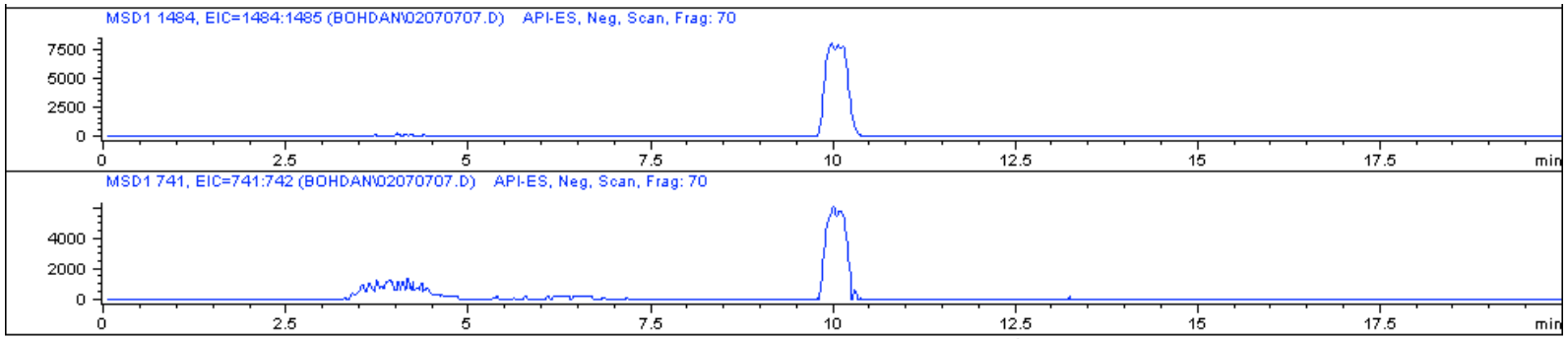
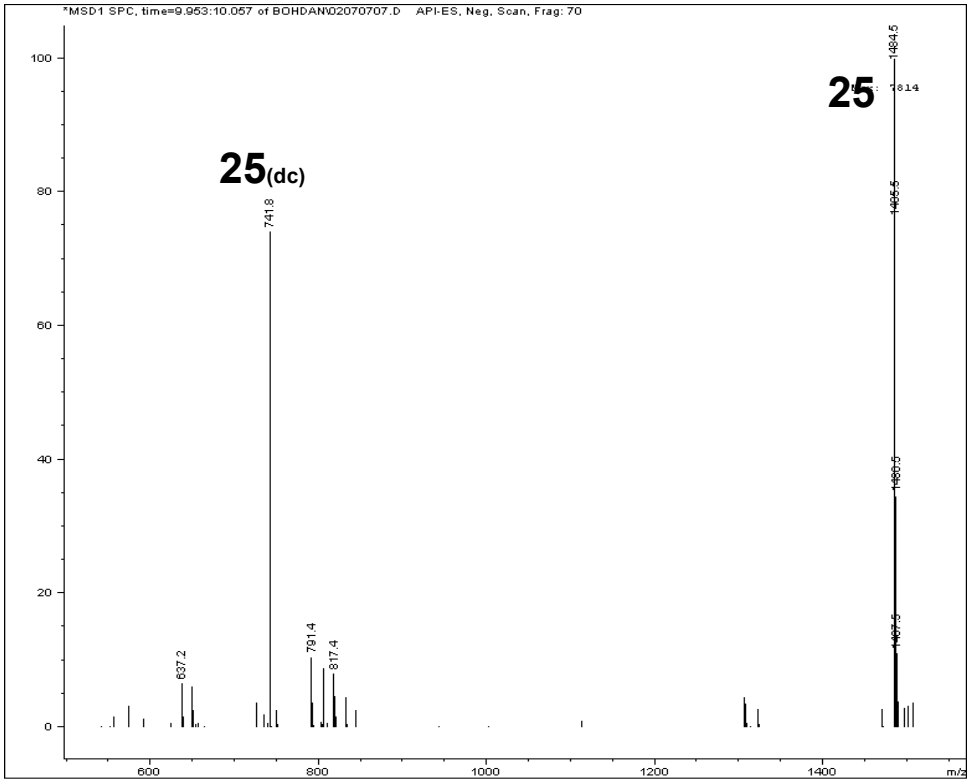


Figure S18. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ*moeA5*Δ*moeB5* strain. **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is **3** (Rt 9.9 min). Peaks corresponding to tetra- and trisaccharide precursors **22** and **20** (as well as their descarbamoylated (d) analogs) are also observed. **3(dc)** is doubly charged ion of **3**. **c.** Exact mass analysis of compound **3**.

a.



b.



c.

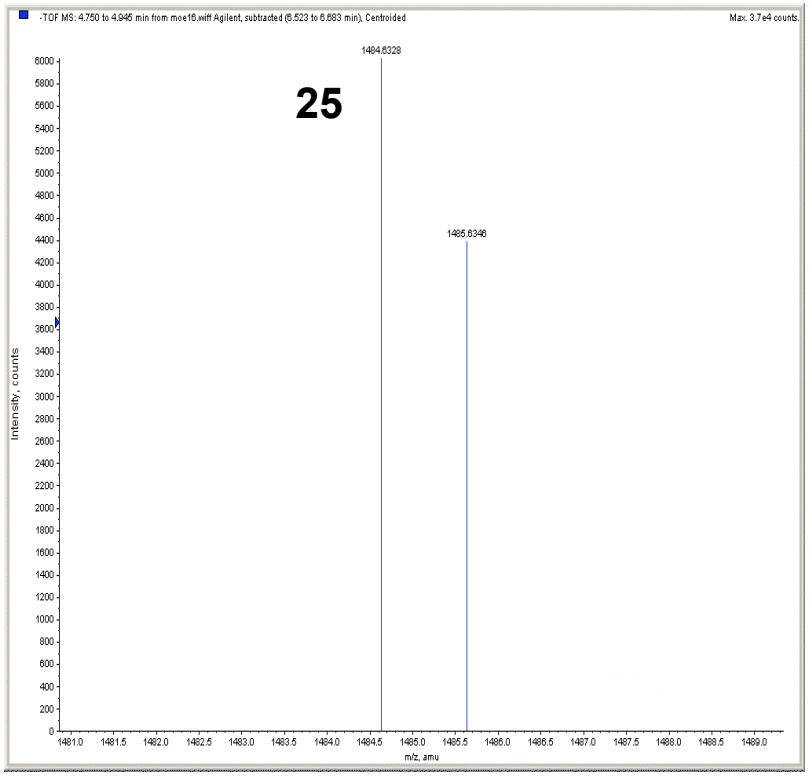
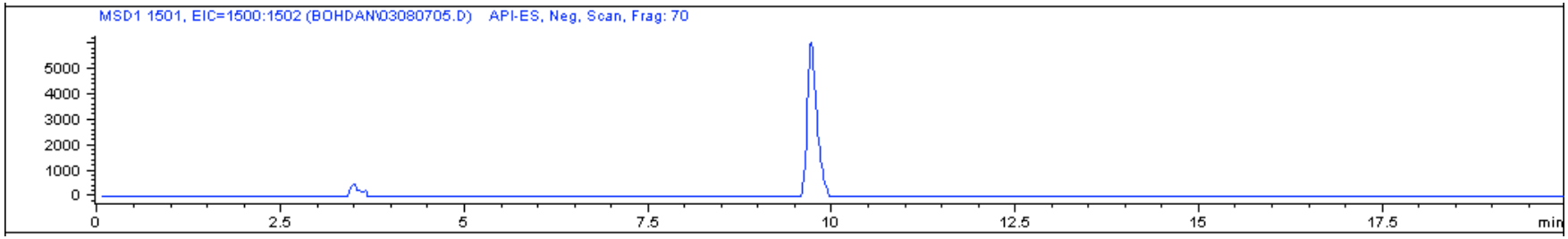
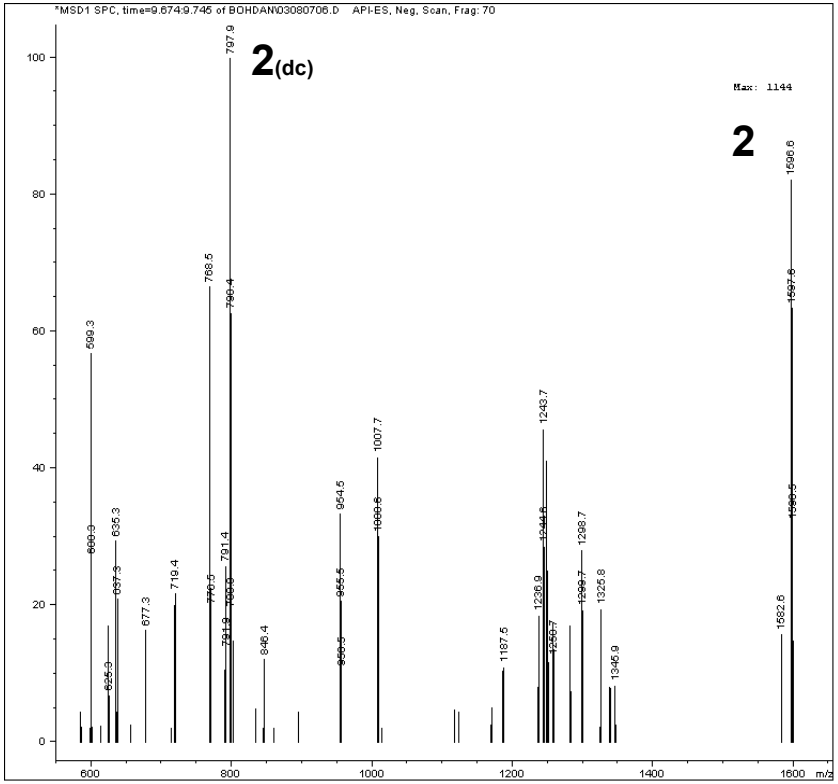


Figure S19. LC-MS analysis of moenomycin metabolites accumulated by the *S. lividans* TK24 Δ*moeA5moeB5* strain overexpressing *moeR5* gene. **a.** Selected ion chromatograms of the purified extracts. **b.** The strain accumulates known compound **25**. **25(dc)** is the doubly charged ion of **25** (Rt 10 min). **c.** Exact mass analysis of compound **25**.

a.



b.



c.

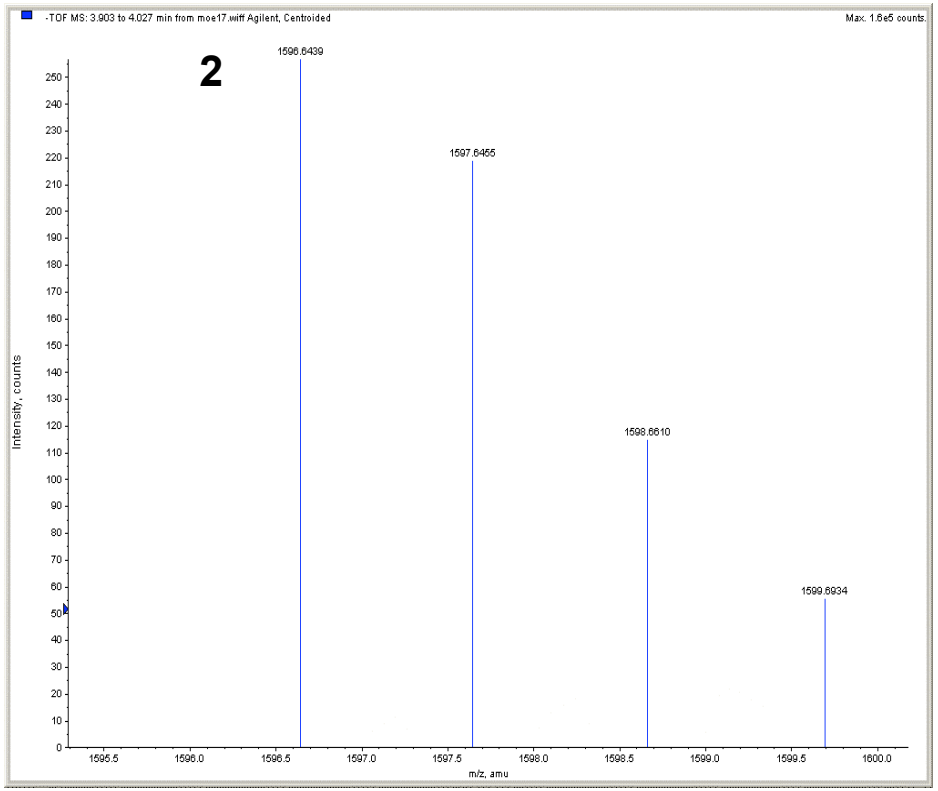


Figure S20. LC-MS analysis of moenomycin metabolites accumulated by *S. lividans* TK24 co-expressing *moe* clusters 2 (pOOB64b) and 1 (except for *moeR5* and *moeS5* genes). **a.** Selected ion chromatograms of the purified extracts. **b.** The final product is pholipomycin (**2**, Rt 9.3 min). **2(dc)** is doubly charged ion of **2**. **c.** Exact mass analysis of compound **2**.

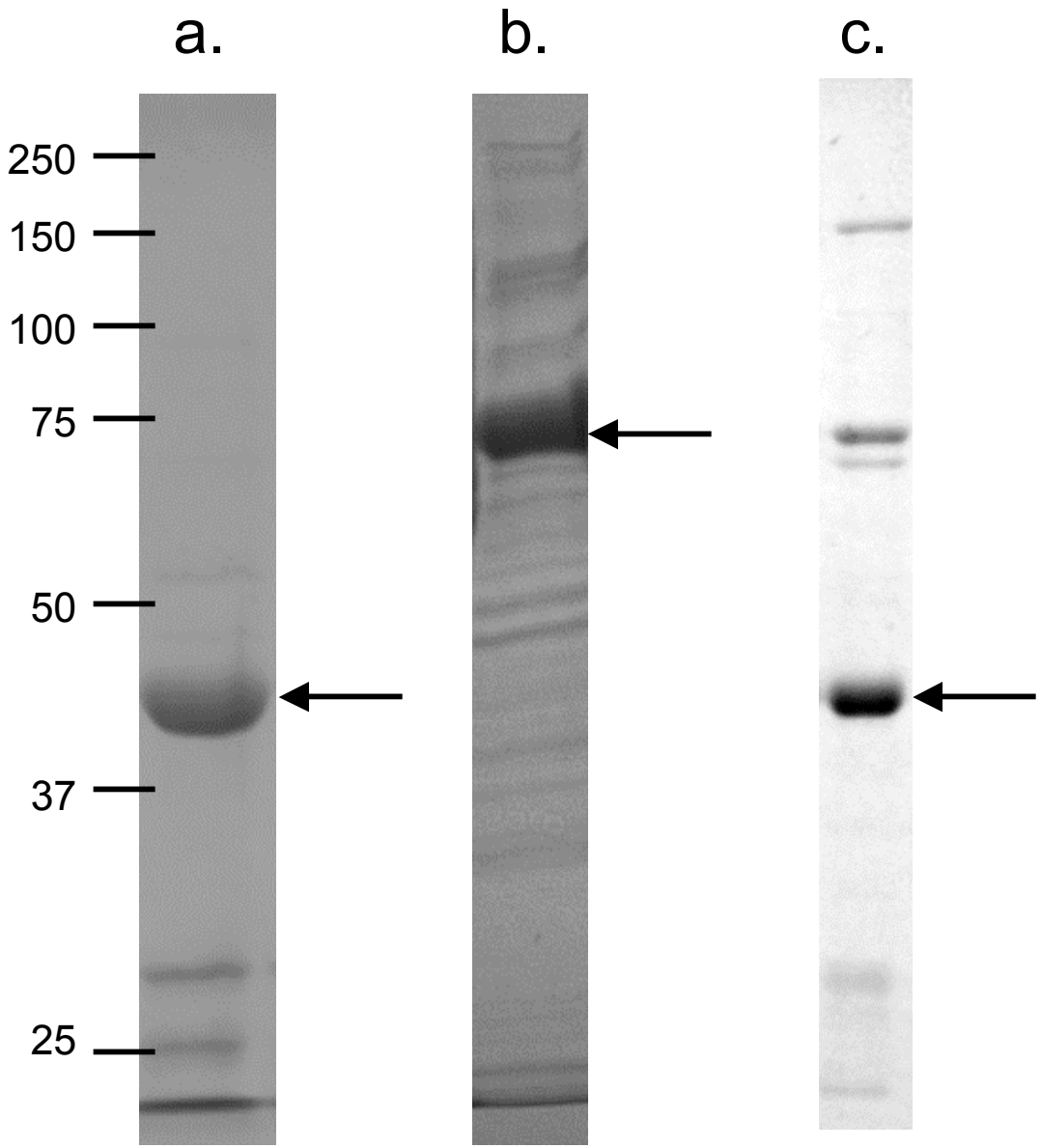
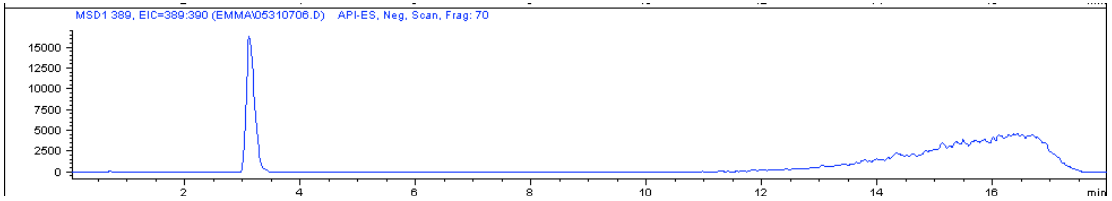


Figure S21. SDS-PAGE analysis of **a**. SUMO-MoeO5, **b**. Trx-MoeE5, and **c**. MoeGT1 following purification via IMAC chromatography as described in Materials and Methods (12% gels).

a.



b.

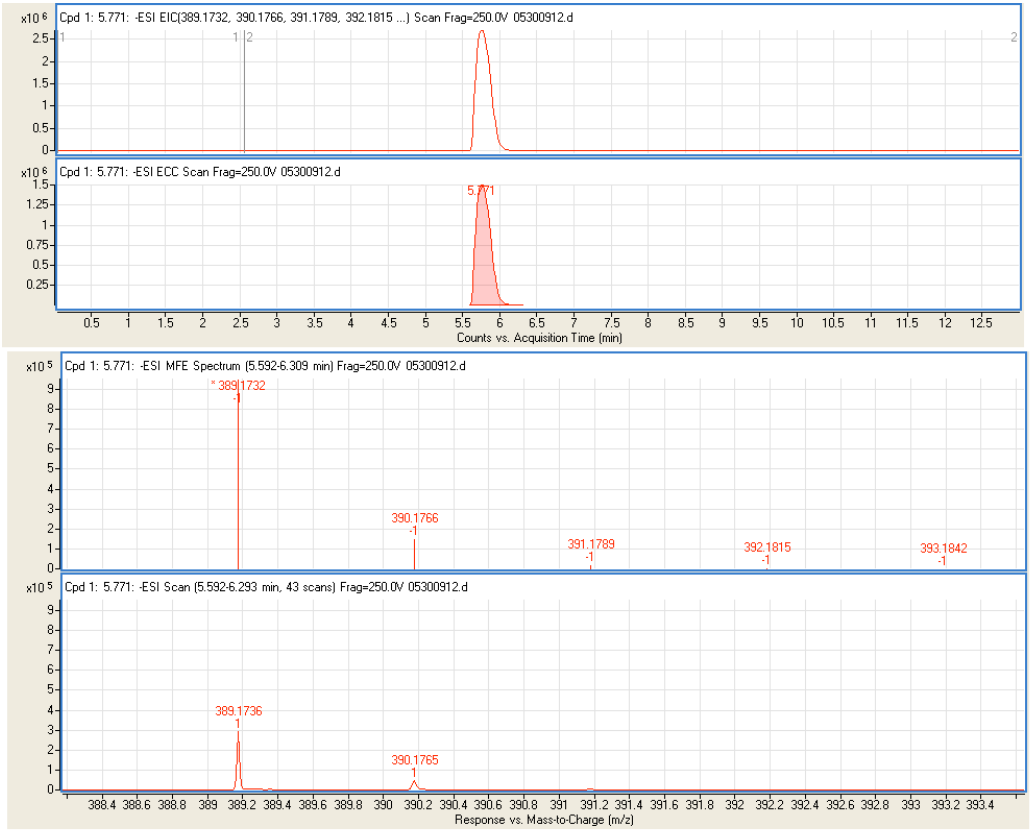


Figure S22. LC/MS analysis of the MoeO5 catalyzed reaction between farnesyl pyrophosphate and 3-D-phosphoglycerate. **a.** Selected ion chromatograms of a 50 µL reaction. **b.** Compound extracted chromatogram from Agilent 6200 and exact mass of compound **7**.

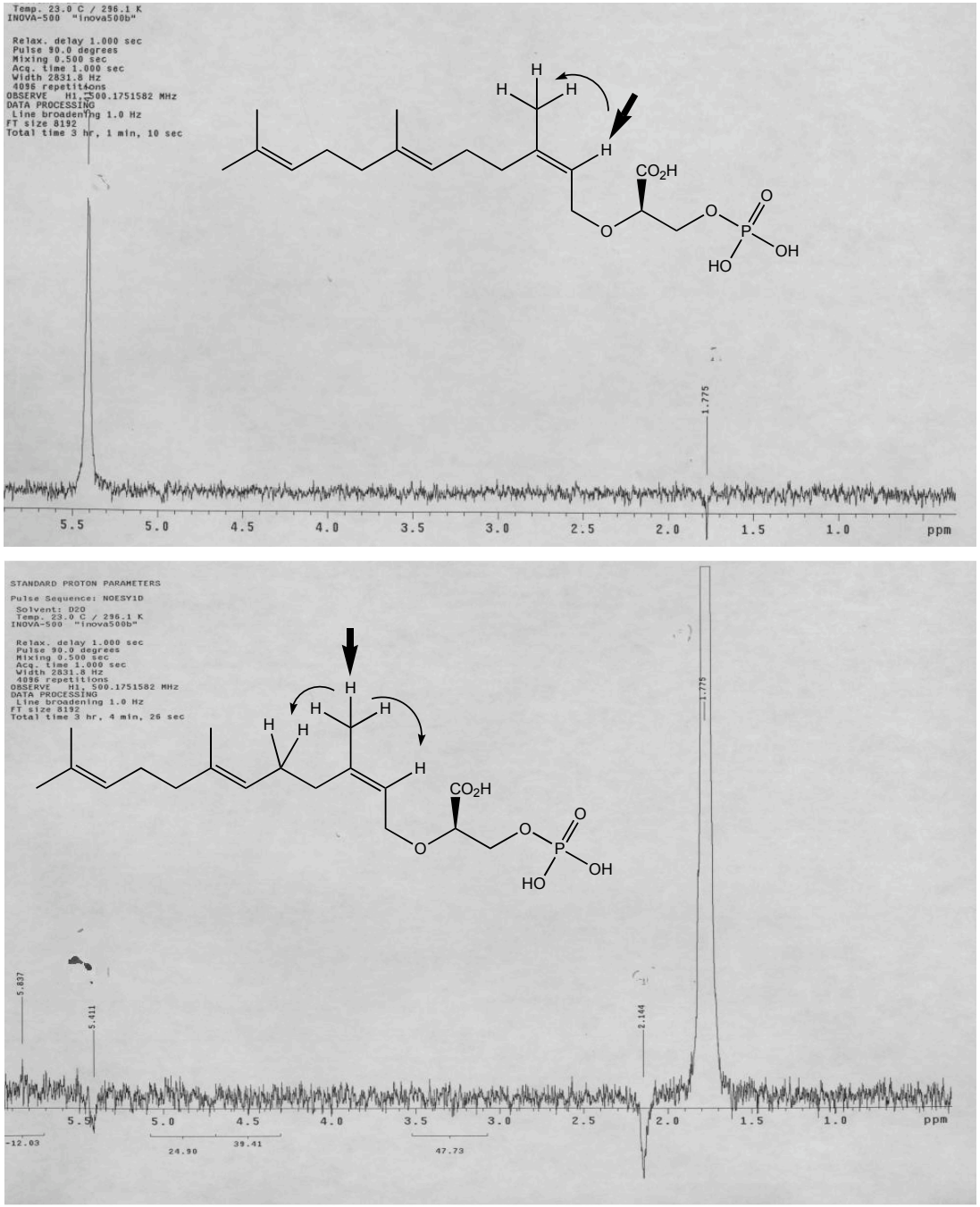


Figure S23. Relevant 1D NOESYs of compound **7**. Interactions are shown.

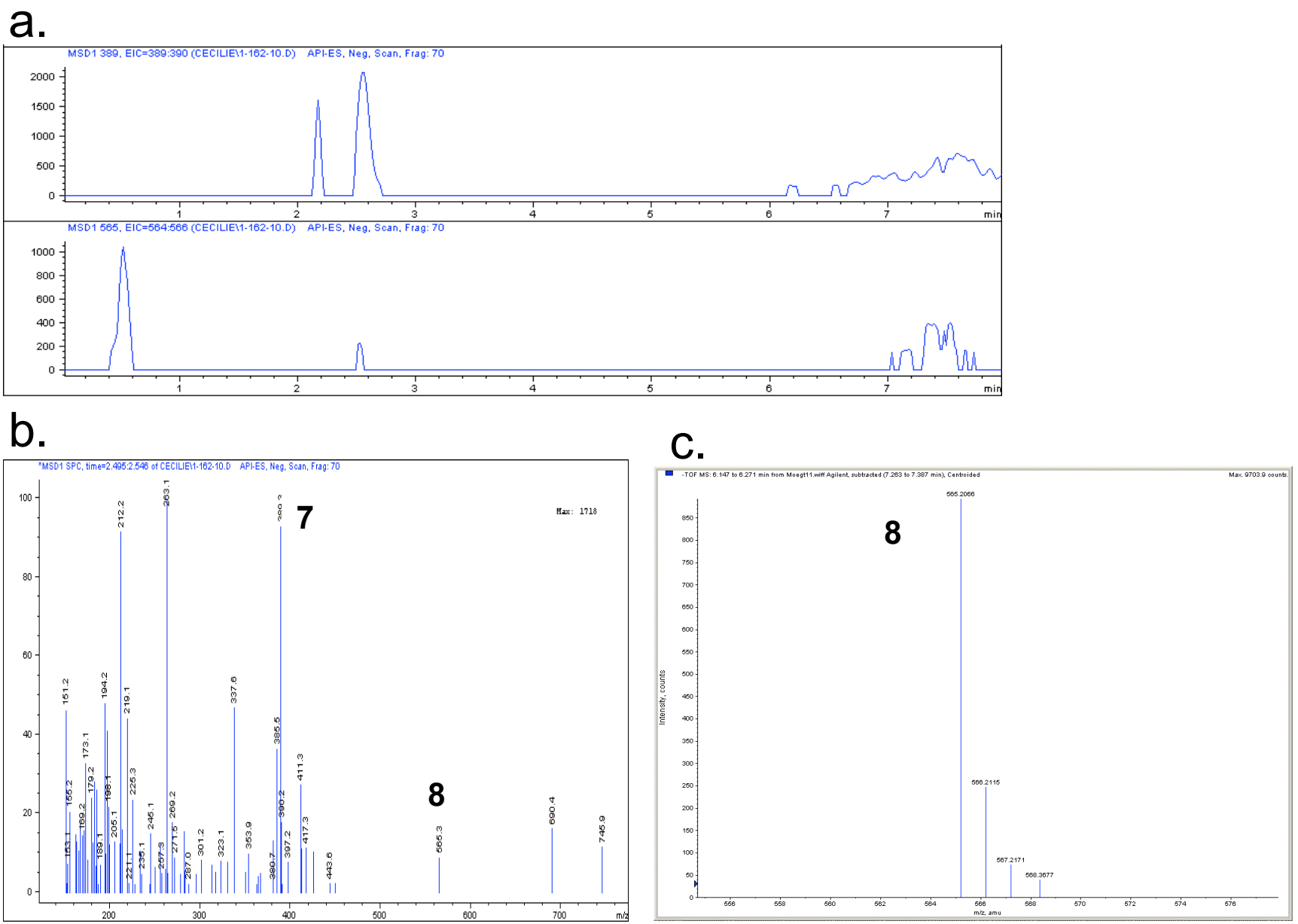
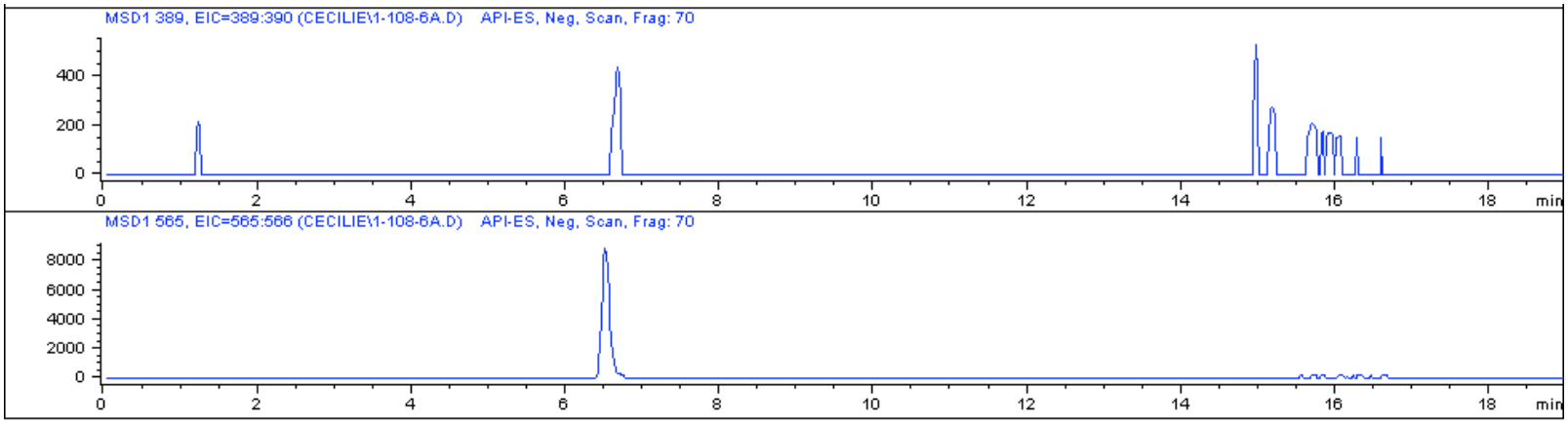


Figure S24. LC/MS analysis of the MoeGT1 catalyzed reaction between compound **7** and UDP-glucuronic acid. **a.** Selected ion chromatograms of a 50 µL reaction. **b.** The final product is compound **8**. Some starting material **7** is also present. **c.** Exact mass analysis of compound **8**.

a.



b.

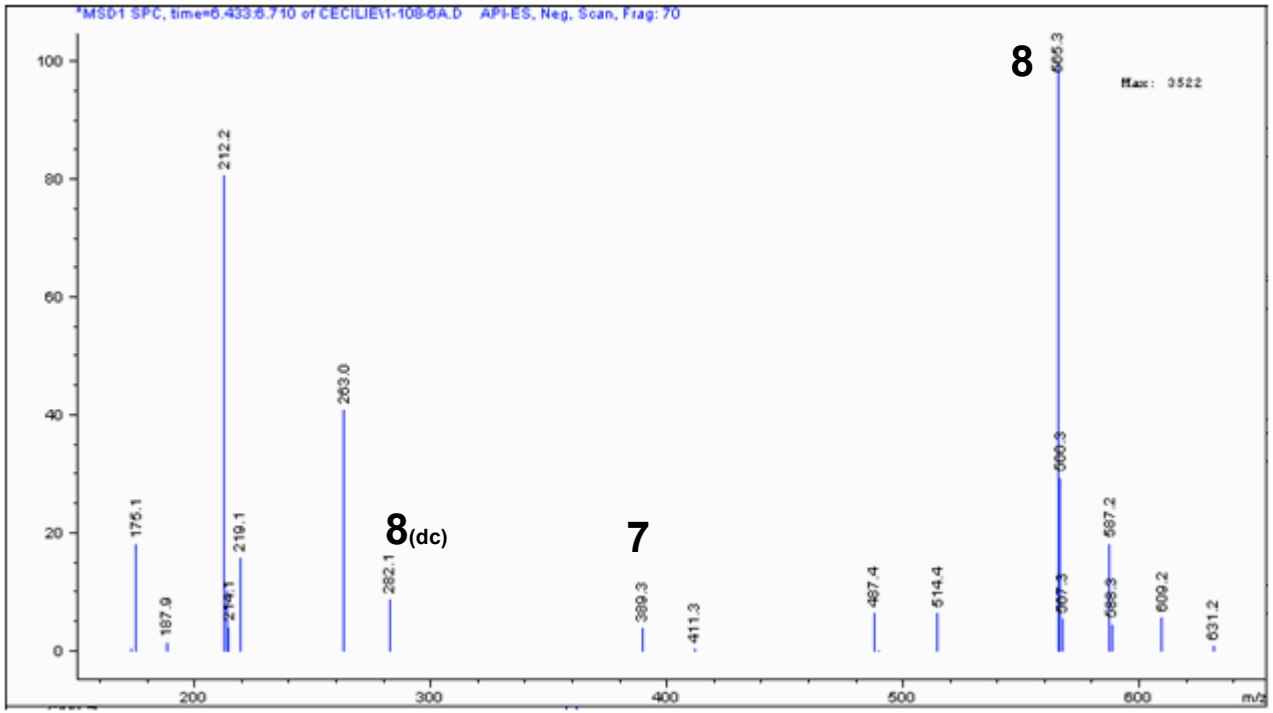


Figure S25. LC/MS analysis of the MoeGT1 catalyzed reaction between compound **7** and epimeric mixture of UDP-glucuronic acid and UDP-galacturonic acid. **a.** Selected ion chromatograms of a 50 µL reaction. **b.** The final product is compound **8**. **8(dc)** is the doubly charged ion of **8**. Some starting material **7** is also present.